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OF THE NAVY ELF COMMUNICATIONS SYSTEM
ECOLOGICAL MONITORING PROGRAM

Volume 1 of 2 Volumes: TABS A-E

July 1984

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Litter Decomposition and Microflora

Bagley, S.; Bruhn, J.; Jurgensen, M.

The Effects of Exposing the Slime Mold Physarum polycephalum to
Electromagnetic Fields

Goodman, E.M.; Greenebaum, B.

Soil Amoeba

Band, R.N.

Soil and Litter Arthropoda and Earthworm Studies

Snider, R.J.; Snider, R.M.

FOREWORD

This document is the second compilation of Annual Reports on the Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program initially authorized under Naval Electronic Systems Command Contract N00039-81-C-0357, and being continued under Contract N00039-84-C-0070. IIT Research Institute, as coordinator for ELF ecology studies, has subcontracted for 10 monitoring projects with several universities and one state agency. This compilation summarizes the activities of those projects from November 1982 through December 1983.

The purpose of the ELF Ecological Monitoring Program is to assess the influence of electromagnetic fields associated with the ELF Communications System on major ecosystem components. Multi-year studies are planned. The first full year of pre-construction studies was completed in Michigan during this reporting period. The 1982-1983 period represents a continuation of operational studies in Wisconsin.

This document was printed from original copies of each principal investigator's annual report for 1983 without change or editing by either IIT Research Institute or the Naval Electronic Systems Command.



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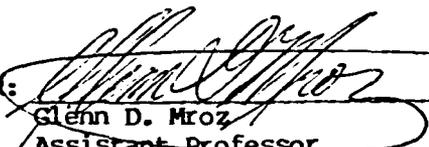
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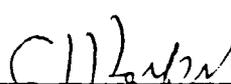
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INTRODUCTION

Since forest vegetation is dominant on the proposed ELF communications antenna area, it is essential to include it in an ecological monitoring program. However, there are several other considerations which justify this study. Trees and herbaceous plants having both above and below ground biomass will be more closely coupled to the ELF field than those organisms solely in the air or on the soil surface (Anonymous, 1977). Trees differ from herbaceous plants in that they are more deeply rooted and are longer lived, while herbaceous plants have been found to be more sensitive to site disturbance than trees (National Research Council, 1977). However, trees offer the unique opportunity to evaluate effects of the ELF electromagnetic field on the same individuals over a much longer period of time while also evaluating changes in stand dynamics. These considerations would be of paramount importance in assessing the significance of ELF field effects occurring at the organismal level.

A secondary consideration is that forest vegetation also exerts strong influence on other organisms within the ecosystem, both above and below ground. These effects include modifying microclimate, exerting influence on soil organisms, particularly in the rhizosphere, and by influencing soil development and fertility through nutrient cycling. By studying the effects of ELF fields on individual plants and plants in the ecosystem, information gained will also be useful to investigators studying other ecological relationships in the ELF environmental monitoring program.

While there are many measures of tree response to any given stand treatment, only a few are generally needed to quantify that response and

test its significance. However, these measurements must be chosen on the basis of highest sensitivity, especially in the case of ELF field effects, since previous work has indicated these may be extremely subtle (National Research Council, 1977). In addition, the measurement must be practical so it can be accomplished as part of a field study outlined for the overall ELF ecological monitoring program. Based on these constraints, the Tree and Herbaceous Plant study has been divided into the following separate elements: (1) plot selection, (2) development, installation, and operation of the ambient monitoring system (3) tree productivity, (4) phenophase description and documentation, (5) herbaceous vegetation cover and growth, (6) mycorrhizal fungi collection, (7) mycorrhizal characterization and root growth, (8) litter production, (9) computer program development and analysis.

There have been changes in study emphasis since our 1982 annual report. These were necessitated by several developments: 1) antenna ground construction will take place earlier than previously planned, 2) certain field procedure will take excessive amounts of time for adequate statistical precision and need to be modified, and 3) the control site was rejected at the end of the field season due to unacceptable differences in levels of 60 Hz fields between the control and treatment sites.

The broad objectives of the study remain: 1) to investigate and characterize the growth of trees and herbaceous plants on selected plots within the ELF antenna area prior to operation of the antenna and 2) use this baseline data to evaluate the possible effects of ELF electromagnetic fields on plant growth. However, results from our investigations over the past 18 months have necessitated a reevaluation and redesign of the original Trees and Herbaceous Plant Cover study plan.

The most major change affecting several elements involves the planting of red pine (Pinus resinosa) seedlings instead of relying on natural regeneration present on the sites. Major reasons for this change include the following:

- 1) Field examination of the sites shows an inadequate number of conifers necessary for ectomycorrhizal root studies.
- 2) We are responding to Michigan DNR concerns on forest regeneration. Since young trees often exhibit a rapid growth rate, possible ELF field effects may be more easily detected.
- 3) Planting at the antenna endpoint as described in the proposals will also alleviate conflicts between study design and construction scheduling by allowing the location of plots before antenna ground installation commences.
- 4) The magnetic fields associated with antenna grounds rapidly decrease in strength over a short distance. Consequently, any plot having a buffer strip of trees along the right-of-way would be too distant from the antenna ground to meet the nominal field strength differences specified by ITRI in the original RFP.

In response to these constraints, we have changed part of the original study to allow the immediate commencement of baseline studies this summer. Instead of working in an established stand, an area will be clearcut along antenna ground and red pine seedlings planted. Once the ground is actually constructed through the planted area, plots will be configured as close as possible to insure maximum ELF field exposure. By establishing a seedling plantation in the cleared area, a buffer strip between the plots and the antenna right-of-way to eliminate edge effect is not needed. For comparison of ELF field effects on fast growing seedlings among treatments, similar plantations will be established at the antenna and control plots.

In order to insure adequate sample sizes for mycorrhizae studies in the pole-sized stands at the antenna and control site, red pine seedlings will also be planted in these stands. While red pine and balsam fir occur naturally over the study area, neither species is represented in the pole-size stands in numbers sufficient for sampling purposes. Studies in the pole-sized stands will proceed as previously planned on the plots established near the antenna and at the control site.

The modifications in study design and plot location have necessitated changing the work schedule and sampling methods of nearly all elements from that presented in the original study plan. Changes which have occurred are described in the following sections.

Element 1. Plot Selection

Tight control over environmental factors is essential to detect possible influences of ELF electromagnetic fields on forest ecosystems. Similarity among test plots and control plots can partially accomplish this by taking into account appropriate site factors that influence vegetation, such as soil characteristics, microclimate and history of the site. Thus, a major portion of the past 12 months has dealt with the selection of treatment and control sites to insure as much as possible, similarity in environmental factors, and the vegetative community.

Initial Plot Location

The Trees and Herbaceous Plants study design requires that plots be located along the portion of the antenna, at the antenna ground, and at a control site located some distance from the antenna. For plots to be established along the antenna ground, its configuration and exact location is required. This was the most limiting criterion plot selection since the antenna ground represents the most limited land area available for study site selection. In addition, the rapid decrease of field intensity in the soil at the grounds necessitates that plots be located as close as possible to the buried wire. Once a study site was selected along an antenna ground, the antenna and control sites had to match the environmental conditions found at the ground study site. Field measurements of environmental parameters for determining similarity among sites are shown in Table 1.

Preliminary investigation of potential study sites was aided by information contained in the Michigan DNR Operations Inventory and Continuous Forest Inventory (CFI) Systems. CFI information showed "upland aspen" to be

Table 1. Field Measurements Used for Describing Potential ELF Study Sites**Trees**

- * Species Composition
- * Basal Area
- * Diameter Distribution
- * Site Index

Ground Flora

- * Species Composition
- * Frequency
- * Crown Coverage

Soil Morphology

- * Horizon Identification
- * Horizon Thickness
- * Texture
- * Drainage
- * Presence or Absence of Earthworms
- * Rock Abundance

Site

- * Slope
- * Aspect
- * Landform
- * Habitat Type

the most common forest type within the ELF antenna area and was tentatively selected for location of the study plots. Approximately 150 CFI plots within the ELF antenna area were identified as the aspen type. However, none of these stands were located within a 1 mile radius of any of the six antenna endpoints. The ground portion of the ELF System will be constructed from each of these antenna endpoints. A detailed reconnaissance of the antenna endpoint areas was then undertaken to locate stands that would be suitable for study.

A stand was identified for possible establishment of plots at the Martells Lake antenna endpoint (T45N, R28W, Sec. 21) through use of aerial photographs in late fall of 1982. In March 1983 this site was selected for establishment of the antenna ground plots. However, when this site was selected, the location and configuration of the buried antenna was not expected to be determined in the near future. Thus, to allow collection of baseline data in this area and to address concerns on the lack of study on forest regeneration, the original study plan was modified as follows:

- 1) Abandonment of detailed studies of overstory and herbaceous vegetation at the buried antenna ground while retaining these studies at the antenna and control plots.
- 2) Replacement of the overstory plot at the antenna ground with a 1.5 ha seedling plantation and establishment of similar plantations at the antenna and control sites. Three replicate plots would be established at each plantation. These plots would be cleared of existing vegetation and planted with red pine (Pinus resinosa) seedlings. This change in experimental design resulted an increase from 9 to 15 plots.

The 1.5 ha plantation was to be located at the point where the antenna meets the ground. This would insure that a sizeable portion of the antenna ground would pass through the plantation. The plantation boundaries were established and site characterization begun. In March 1983 we were informed by IITRI that the antenna endpoint location had been moved several hundred feet to the northeast. Previous work and plots were abandoned and a new study site established at the revised antenna endpoint in April. During March and April of 1983, with the assistance of DNR personnel and aerial photography, a number of possible antenna sites and control sites were identified. These stands were subsequently visited and substantial field measurements were taken to determine their suitability as study sites. Based on this data, the antenna and control locations were selected and three replicate plots were established at each study site (Table 2). Actual site locations and plot configuration are given in Appendix A.

Vegetation Characteristics

Vegetation at each study site represents an Acer-Quercus-Vaccinium habitat type. A summary of AQV habitat type characteristics appears in Appendix B, while concepts and complete details of the habitat type system can be found in Coffman et al. (1983). Detailed tree and soil data from each site was collected and analyzed to further quantify the similarity between sites. Methods and results are presented in the following sections.

Tree Inventory

A 100 percent inventory of all trees was made on each plot. Total tree height, DBH (diameter at breast height) and insect and disease damage were recorded for all trees greater than 2.5 cm DBH. A summary of DBH and height data appear in Table 3 and are further discussed under Element 3: Tree Productivity.

Table 2. Description of ELF Antenna Study Sites

Location	Antenna Endpoint (Ground) T45N R29W Sec. 21	Antenna T45N R29W Sec. 28	Control T41N R32W SW 1/4 Sec. 4
Percent Slope	0-30%	7-15%	10-15%
Aspect Range	W-N	W-NW	NW
Slope Position	All positions represented	Crest to mid-slope	Mid-slope
Elevation	445 M	454 M	408 M
Habitat Type	Acer-Quercus-Vaccinum	Acer-Quercus-Vaccinum	Acer-Quercus-Vaccinum

Table 3. Mean Tree Diameter and Height at ELF Antenna Study Sites

Species	Antenna Endpoint			Aboveground Antenna			Control		
	DBH(cm)	Ht(m)	n	DBH(cm)	Ht(m)	n	DBH(cm)	Ht(m)	n
Bigtooth Aspen	21.2	19.9	26	22.8	19.0	14	19.7	20.0	109
Sugar Maple	7.8	12.5	1	-	-	-	-	-	-
Northern Red Oak	21.8	19.1	55	19.9	15.4	66	19.5	18.2	73
Paper Birch	15.1	16.6	81	19.2	18.9	8	13.6	15.2	55
Quaking Aspen	22.6	18.8	20	-	-	-	-	-	-
Red Maple	9.5	12.0	272	12.6	13.9	207	8.0	10.4	164
Red Pine	23.1	13.0	4	11.5	7.8	1	7.5	5.8	13
White Pine	36.7	20.5	1	2.9	3.1	2	6.2	5.8	34

Several similarity indices were applied to the tree inventory data to estimate similarities now existing between the three sites (Mueller-Dombois and Ellenberg, 1974). The presence/absence of tree species was quantified using the Jaccard and Sorenson similarity indices where

$$\text{Jaccard: } IS_j = \frac{\text{common species}}{\text{all species}} \times 100$$

$$\text{Sorenson: } IS_s = \frac{\text{common species}}{1/2(\# \text{ species in one} + (\# \text{ species in another population)})} \times 100$$

Similarity between the sites based on total tree biomass of each species was quantified by the Ellenberg similarity index where

$$\text{Ellenberg: } IS_E = \frac{C/2}{A + B + C/2} \times 100$$

and

- C - the sum of percent biomass of common tree species
- A - the sum of percent biomass unique to population A
- B - the sum of percent biomass unique to population B

Results from these indices can be found in Table 4. Sorenson's index differs from Jaccards in that it gives greater weight to the species that recur in the two test areas than to those that are unique to either area; concern is based on the similarities between the sites and not on the uniqueness, thus Sorenson's index was given greater emphasis. The indices indicate a strong similarity (86%, 93%, and 100%) between the sites.

Table 4. Overstory similarity indices.

Comparison	<u>Percent Similarity Among Plots</u>		
	Jaccard	Sorenson	Ellenberg
Antenna endpoint & Antenna site	75	86	93
Antenna endpoint & Control site	75	86	93
Antenna & control site	100	100	100

Changes in Plot Location

In July, 1983, a map was received from IITRI describing the tentative configuration of the antenna ground near Porterfield Lake Road. This map showed a feedline traversing our proposed plantation from the antenna endpoint to the antenna ground. In a late summer update we were informed that the line would traverse our study site and that our study site should be relocated. It was suggested that it be located near the wells planned for the buried antenna, but this was not possible due to extreme differences in plant cover type.

We immediately began to look for suitable study sites along the antenna ground route as shown on the July map. A preliminary survey of the route was made during the spring of 1983, however, little evidence of the survey line remained, making reconnaissance difficult. A site similar to the antenna site and the control site was located south of the antenna endpoint. Little site characterization work was done here due to the tentative nature of the survey line. In mid-October, the actual survey of the buried antenna was completed. This line is approximately 100 feet north of the preliminary survey line (see maps Appendix A). Fortunately, the desired forest stand was large enough to accommodate the difference in location between the preliminary and actual survey lines. Site characterization work was started, but due to the late season only the soil description was completed.

Also, in November 1983, we were informed by IITRI that our established control site (4C2) was unsuitable because background 60 Hz electromagnetic fields at this location differed by more than one order of magnitude from the 60 Hz field at the antenna (4T2) and antenna endpoint (4T1) sites.

Soil Characterization

Complete pedon descriptions and samples were obtained from soil pits at the antenna, buried antenna, and control sites. These profile descriptions and laboratory data reports are presented in Appendix C.

At each treatment site reconnaissance investigations were conducted by making auger borings to 1.5 meters and noting a profile description. This was done to assess the uniformity of soil conditions across the treatment site. A soil pit was excavated at a representative location adjacent to the study plots and a detailed profile description made according to National Cooperative Soil Survey Standards. Bulk density samples and soil samples for physical and chemical analysis were obtained for each soil horizon. Composite samples were obtained from each plot for the upper 4 mineral soil horizons, by extracting five individual cores from a 2m² area. Three sets of composites were sampled from each plot. No composite samples were obtained from the buried antenna plots since they have not been established. Each of these samples were returned to the Soil Research Laboratory at Michigan Technological University for physical and chemical analysis. Results are summarized in Appendix D.

Standard analytical techniques (Soil Conservation Service, 1972) were utilized for the chemical and physical analyses. Complete analyses were performed on the pit soil samples, while only selected chemical analyses were performed on the composite samples. The specific analyses for each pit soil sample are presented in Table 5.

Table 5. Chemical and Physical Analyses of Soil from ELF Antenna Study Plots

<u>Physical Analyses</u>	<u>Chemical Analyses</u>
Particle Size Distribution	Ca, Mg, Na, K, Fe, Al,
Moisture Retention	pH, CEC, total N, C,
Bulk Density	H

A synopsis and complete reference of the laboratory procedures are presented in Appendix E.

The three antenna study sites are similar in overall soil characteristics. All have sandy textures, low water retention capacities, thin surface layers containing high levels of nutrients, and infertile subsurface layers. The pH values of the surface layers vary from 4.1 at the overhead antenna site to 5.3 at the ground site, while the C horizons vary only from 6.1 to 6.5.

As noted earlier, the control area sampled will not be used. There are some minor differences in soil properties between the antenna site and the ground site. Although the soils are similar texturally, with layers containing high levels of fine and medium sand overlying stony layers of coarser sand, the depth at which the coarser sand occurs is 67 cm on the antenna site and 92 cm on the buried antenna site. This small textural difference, in combination with slightly lower levels of iron and aluminum at the ground site, resulted in different classifications on the two sites. Surface horizons also differ slightly between the two sites, with those at the antenna site containing greater amounts of carbon and nitrogen and having higher cation exchange capacity. Subsurface horizons are chemically very similar. These slight soil differences between these sites is probably not sufficient to affect the monitoring of ELF field effects.

Work is underway to locate a new control site. Plot screening is proceeding through the use of remote sources such as aerial photography and soil survey maps. However, similarity based on herbaceous plant cover will be delayed until these plants appear in June 1984. Verification of potential control sites will continue through the winter, but it is dependent on field accessibility.

Element 2. Ambient Monitoring

The terrestrial ambient monitoring program is designed to provide for the collection and analysis of climatological and soils data which affect plant growth processes. These parameters include precipitation, air temperature, relative humidity, solar radiation, soil temperature and soil moisture. In addition, soil characterization studies are also being conducted on each study area to evaluate the compositional and morphological properties of the soils. The ambient monitoring element is being conducted to support other research elements within the tree, herbaceous and litter decomposition tasks. Accordingly, the ambient monitoring program has maintained a structure and design which is consistent with the data requirements of our entire research program. The appropriate elements should be referred to for discussions regarding changes in the overall plot design and site selection processes.

System Configuration

The original proposal (June, 1982) provided for the collection of ambient data on each treatment site, i.e. antenna, ground and control. Each of the three study plots within a site were to be instrumented, resulting in a total of nine plots. Subsequent redesign of the overall study to include the red pine regeneration research has resulted in 15 study plots within the three treatment sites. This design change has resulted in a reconfiguration of the ambient monitoring system. The resulting changes still provide adequate information on relative humidity, air temperature, precipitation, incoming solar radiation, and soil temperature and moisture levels within the rooting zone.

One data collection platform (Handar Model 540A) will be used for each study site for data collection, summary and transmission of the NESS (National Earth Satellite Service) GOES East Satellite. This data is retrieved at the Wollops Island ground station and stored in the Ness Computer until retrieved by MTU. Sensor data will be collected at 15 minute intervals and summarized for computation of a 90 minute average. This data for each sensor channel will be transmitted every 3 hours.

Data collected at each treatment site will include precipitation (rain and snow), air temperature, relative humidity, soil moisture, soil temperature and solar radiation. Air temperature measurements will be taken at 1.0 meter above the soil surface. Soil temperature and moisture measurements will be taken at depths of 2 cm and 10 cm on every plot; and additional depths of 25, 100 and 200 cm on one plot each at the antenna and the control. The specific sensor configuration for each treatment site and study plot is presented in Appendix F.

Progress

The ambient monitoring system was purchased from Handar, Inc., Sunnyvale, California. All equipment, except 29 soil sensors and 2 snow pillows, have been received.

Program and calibration of the data acquisition units has been initiated. This phase consists of writing individual programs for each sensor channel, on the data collection platform. These programs provide for sensor interrogation, data manipulation, storage, and transmission to the GOES satellite. All data will be summarized at 90 minute intervals, with collection rates ranging from 2 to 15 minutes. Air temperatures will be reported as a 90 minute mean value with the association minimum and maximum during the period. Soil

moisture, soil temperature and relative humidity will also be reported as a 90 minute mean value. Precipitation and solar radiation data will be accumulated during the period and reported as a 90 minute total amount. The soil moisture sensors are being calibrated for the soil horizons on each of the study plots.

Data Communication and Storage System

The University has entered into a Memorandum of Agreement with the National Environmental Satellite, Data, and Information Service (NESDIS) to utilize the GOES East Satellite and the NESDIS downlink communication network to transmit ambient monitoring data from the ELF sites to the Michigan Tech campus. The period of the agreement is 10 years and may be renewed. Required applications have also been made to the Federal Communications Commission to secure transmit approval over our assigned frequency of 401.7130 MHz. This is a routine application procedure for new transmitting facilities and will be valid for the duration of the project.

The ambient monitoring data transferred via satellite to NESS must be retrieved and transferred to Michigan Technological University UNIVAC for storage into the SIR data base on a daily basis. A procedure to accomplish the data transfer was designed, based on conversations with NESS personnel, University computer systems staff and NESS users currently doing data transfer. The design used the University VAX 750 Computer to transfer the data between NESS and MTU over a 300 baud asynchronous dialup phone line. Once the data had been stored on the VAX, it is then transferred over a 1200 baud asynchronous dialup phone line to the University UNIVAC 1100/80 for storage in the SIR database.

Arrangements had been made to use the VAX computer. Ports on both the VAX and UNIVAC were rented on a monthly basis and the necessary modems purchased. Design on the VAX programs necessary to control the data transfer between NESS and the VAX computers, and the VAX and UNIVAC computers had been initiated.

While designing the control programs, it became apparent that the multi user environment on the VAX presented more problems than originally anticipated. Rather than spend the extra time and computer costs trying to solve the problem with the multi user environment on the VAX, it is now possible to do the data transfer using an IBM Personal Computer. The IBM PC and associated software was not available at the time the data transfer process was originally designed. Using the PC would greatly simplify the data transfer and the costs associated with using a PC would be equivalent to the costs of using the VAX. As a result, design of the VAX control programs has been suspended while the IBM PC is investigated further. The final system configuration will be completed in January, 1984, and system testing will be conducted in conjunction with tests of the DCP.

Element 3. Tree Productivity

Tree growth is sensitive to environmental disturbances. To reflect any changes in the growth due to these disturbances, accurate tree measurements need to be recorded. The most widely accepted tree growth measurements are diameter at breast height outside bark (DBH) and height; of these two variables, height is the more difficult to measure. The installation of permanent dendrometer bands on the stem of a tree would allow measurement of minute changes (0.254mm) in diameter over a short time interval (Husch, Miller and Beers, 1972). Two additional advantages in using DBH as a measurement of tree growth are the responsiveness of cambial activity to environmental effects (Smith, 1962) and the strong correlation existing between DBH and total biomass of the tree (Crown, 1978). Consequently, measurements of diameter increment will be the primary response variable and height will be used as support in stand characterization data.

While DBH and height measurements can provide information on present stand production and a means to predict future productivity, the capacity of a stand to continue producing can be determined by monitoring tree reproduction and mortality. Stand structure, the distribution of trees by diameter classes, changes from year to year due to natural ingrowth (reproduction) and mortality of trees. Any environmental disturbances could produce an effect on these two factors; thus, natural changes need to be monitored and recorded in order to distinguish these from changes produced from disturbances. Therefore, to achieve a complete picture of site disturbance on tree and stand production, DBH, height, ingrowth, and mortality all need to be measured.

Progress

A 100 percent inventory of all trees with a DBH greater than 2.5 cm was conducted at each of the three sites. Tree species, total height, DBH, and insect and disease damage was recorded for each tree. Diameters were measured to the nearest 0.1 cm using diameter tapes and heights were measured to the nearest 25 cm with a Sunto clinometer. From these measurements basal area and total tree biomass were calculated; the regression equations used in estimating total tree biomass for each tree species can be found in Appendix G. A summary of the inventory data is found in Table 6.

At this point, with only first year measurements of DBH available, no diameter growth information can be calculated or analyses conducted. The information collected this year provided an outline of stand structures. In the following years, analyses will be conducted on diameter growth data. Figures 1, 2, and 3 summarized the stand structure by 1 cm diameter classes, existing on the respective sites this year. The present mean DBH and standard deviation about the mean of two trees at the endpoint, at the antenna and at the control were 13.38 cm and 7.42 cm, 14.59 cm and 7.52 cm, and 13.25 cm and 6.87 cm, respectively.

A Kolmogorov-Smirnov two sample test tested the hypothesis of similar diameter distributions existing on the aboveground antenna site and the control site. Incorporating DBH measurements from the inventory this test rejected the hypothesis of similar diameter distributions at the $\alpha = 0.05$ level. As seen in Figures 1 and 2, the distribution found on the antenna site appeared unimodal with most trees found in the 11 cm class whereas the control site the distribution appeared bimodal with most trees found in the 6-8 cm class and the 18 cm class. Also, the average basal area per tree was

Table 6. Summary of 100 Percent Tree Inventory on Tree and Herbaceous Plant Cover Study Plots.

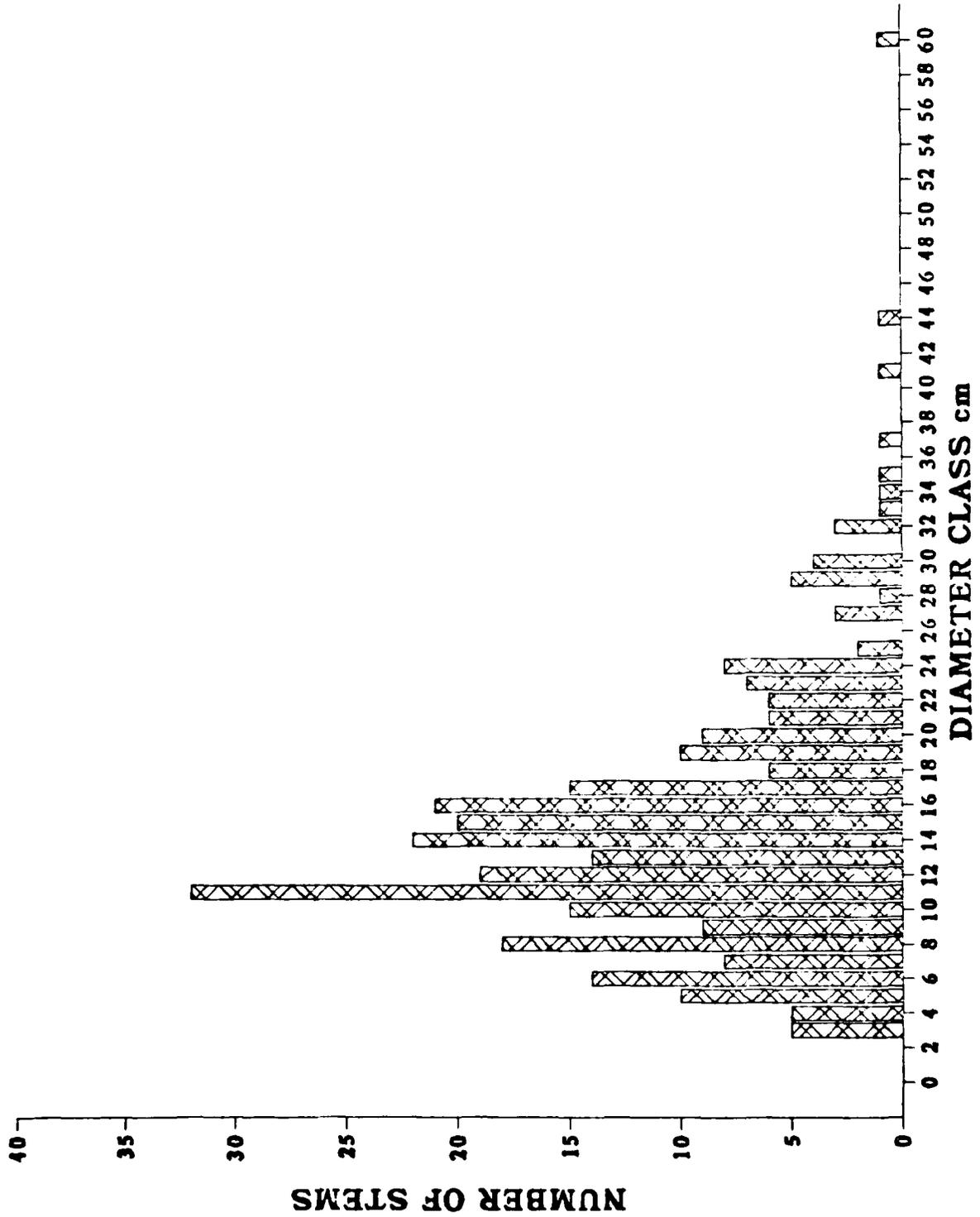
Species	(kg/ha) ² Biomass			(m ² /ha) Basal Area			Number of Trees/ha			Average DBH (cm)			Average Ht (m)		
	1 ¹	2 ¹	3 ¹	1	2	3	1	2	3	1	2	3	1	2	3
Bigtooth Aspen	1131.07	636.69	4487.90	3.17	2.02	10.84	82	44	346	21.1	22.8	19.7	19.93	19.02	19.98
Hard Maple	205.01	-	-	0.01	-	-	3	-	-	7.8	-	-	12.50	-	-
Jack Pine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Misc. Species	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N. Red Oak	541895.30	744028.51	563488.23	6.89	8.30	7.44	174	209	231	21.8	19.9	19.5	19.12	15.45	18.15
Paper Birch	4572.16	576.08	2778.09	5.46	0.78	2.80	257	25	174	15.1	19.2	13.6	16.62	18.88	15.19
Quaking Asper ²	24915.27	-	-	2.75	-	-	63	-	-	22.6	-	-	18.80	-	-
Red Maple	95929.39	134999.28	38574.75	7.21	9.18	3.10	862	656	520	9.5	12.6	8.0	11.96	13.92	10.37
Red Pine	21143.30	382.85	3472.16	0.97	0.03	0.28	13	3	41	23.1	11.5	7.5	13.0	7.75	5.75
White Pine	77.43	16.34	542.07	0.34	0.003	0.38	3	6	108	36.7	2.9	6.2	20.50	3.13	5.77
TOTAL	689868.91	880639.74	616193.18	26.80	20.313	24.84	1458	945	1420						

¹1 denotes the ground, 2 denotes antenna plots, 3 denotes the control plots.

²2 equations predicting whole tree biomass for each species are found in Appendix G.

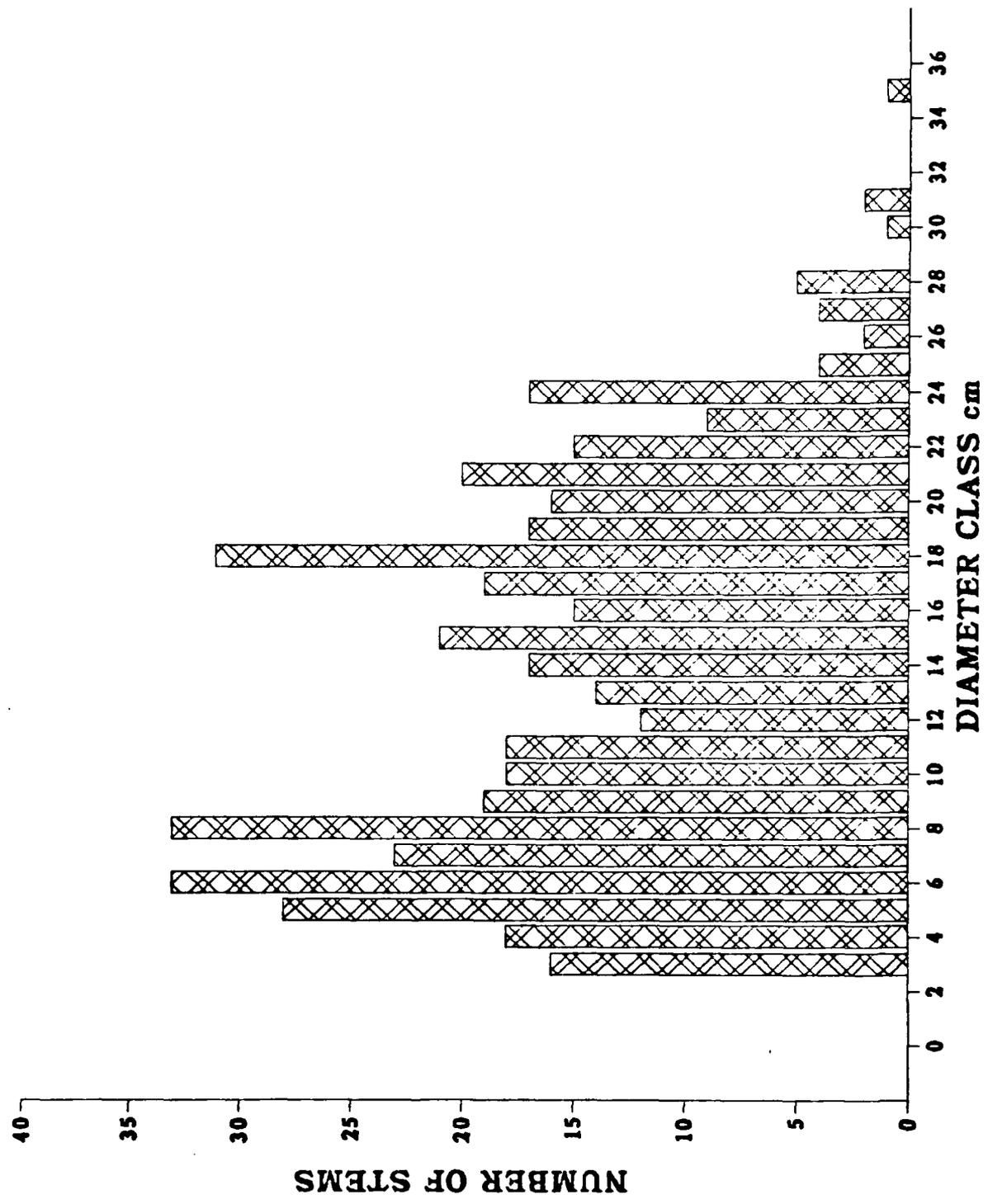
DIAMETER DISTRIBUTION ABOVEGROUND ANTENNA SITE 1983

Figure 1.



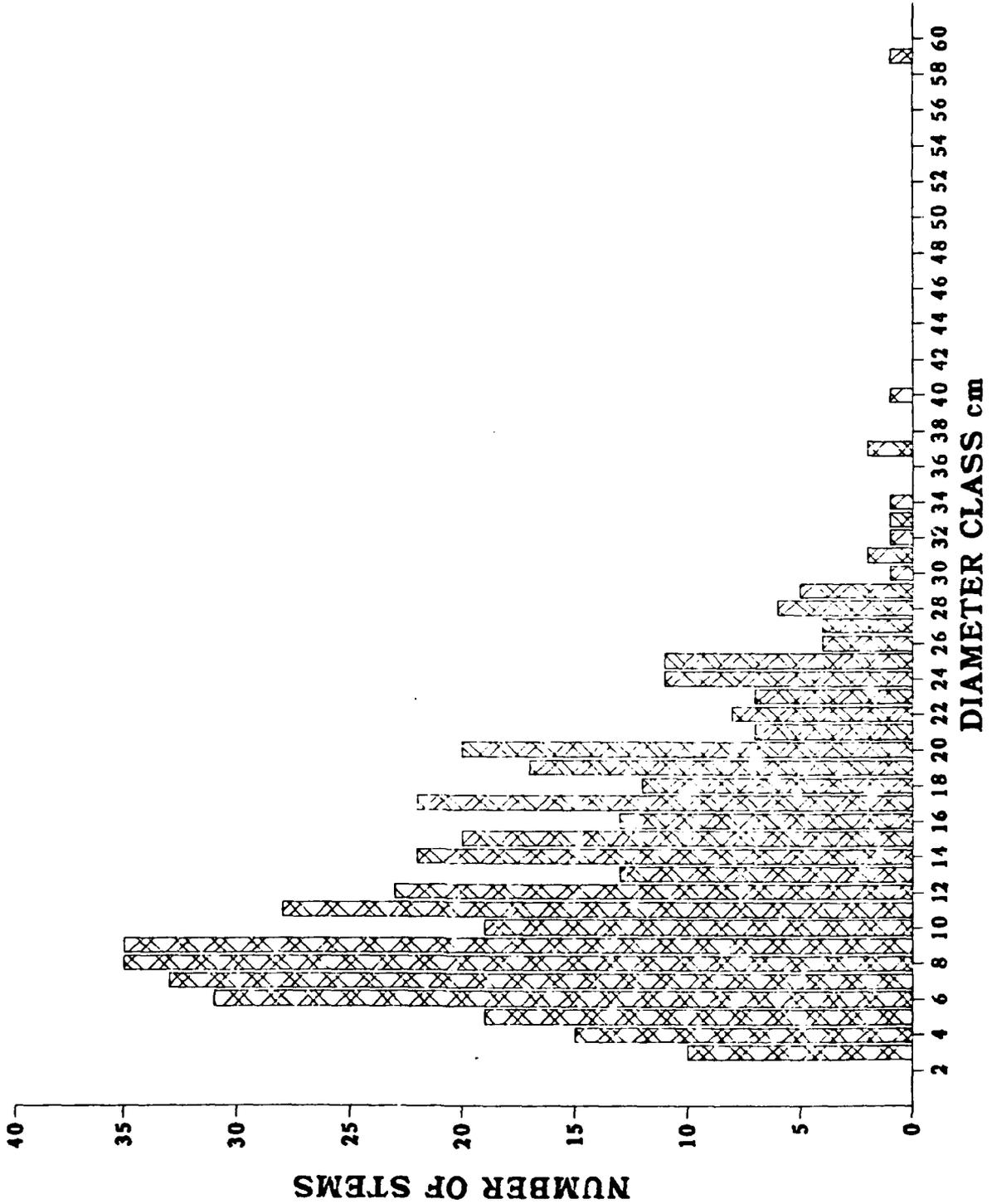
DIAMETER DISTRIBUTION CONTROL SITE 1983

Figure 2.



DIAMETER DISTRIBUTION ENDPOINT SITE 1983

Figure 3.



greater $(0.022)m^2$ for the aboveground site than for the control $(0.017)m$. All of this indicated that with larger trees located on the antenna site, the diameter growth increment would be smaller than the growth increment on the control site and possible changes in these increments due to site disturbance might be hard to detect unless they were more comparable. If there was no possibility of achieving similar distributions, the analysis might compensate for this fact using DBH and stand density as covariates or a selected subgroup of similar sized trees on each site will be measured over time. However, it would be more advantageous in detecting changes to work with similar distributions. Since the control site is to be discarded, these considerations will be made in the selection of the new site.

Measurement of diameter growth is to be obtained from dendrometer bands; the number of trees per diameter class that need to be banded was determined from the present inventory data. This number calculated was to best estimate diameters, not diameter growth. No growth data (second year measurements) are available now. To put dendrometer bands on trees as soon as possible the required number of trees that needed to be banded in order to estimate diameter on a site within a given bounds of error is given by the following formula (Cochran, 1977):

$$n = \frac{t_{n-1, .05}^2 s^2}{E^2}$$

where

- t_{n-1} = the value of the student's t-statistic at a desired α -level with $(n-1)$ degrees of freedom
- s^2 = the estimated variance of the sample diameters
- E = the bounds within which estimate of diameter was desired

An acceptable bounds of $E = 0.5$ cm was selected and at an α -level of 0.05, t was approximately 1.96. One is 95% confident of detecting changes in diameter at breast height of 0.5 cm. However, estimated variances of DBH in 10 cm diameter classes and above were so high on both sites (antenna and control) that they warranted banding all trees in all diameter classes on both sites. By doing this, measurement error is eliminated and the actual diameters will be known, but until growth data are available, no estimate of the precision or variance of growth changes can be estimated. When the growth data is measured and analyzed, required sample sizes (number of trees needed to be banded) can again be calculated by species and these values may change.

At this time approximately 35% of the trees on the antenna site have dendrometer bands on them; the remaining 65% will be banded before the growing season begins. These bands will be checked weekly until the growing season has begun and subsequently measured and recorded approximately every 2 weeks. Respective total tree heights will be measured and recorded annually.

Element 4. Phenophase Description and Documentation

The timing of periodic biological events in plants is often referred to as phenophases. These include initial or full flowering, leaf out, bud burst, leaf fall, etc. Measurements of phenophases are of value in an ecological monitoring program because they are indicators of physiological processes that are affected by environmental inputs. Therefore, phenological changes would indicate physiological changes. Plants, then, can be considered to integrate and express the net effect of environmental variables, such as possible effects due to ELF electromagnetic fields.

Phenological observations are subjective in nature thus, precise descriptions of each phenophase identified for study needs to be documented. This can be accomplished by compiling a photographic record of specific developmental stages to be used as standards that will insure year to year accuracy.

Progress

Since the study site at the antenna ground will be cleared of existing vegetation prior to planting with red pine, hardwood phenology measurements will be obtained only on the pole-sized tree plots at the antenna site and control site. The following phenophases will be recorded for hardwood species on these plots: bud burst, leaf out/full foliage, flowering, cambial activity, seed dissemination, and leaf fall.

Originally trembling aspen, bigtooth aspen, sugar maple, paper birch, and balsam fir were to be studied. However, when the sites were selected (see Element 1: Plot Selection), trembling aspen, sugar maple, and balsam fir were not found in sufficient numbers for study. Red maple and red oak are present

on these plots and, together with bigtooth aspen and paper birch, will be used for phenology work. Phenological events for red pine seedlings will be documented on the planted sites at the antenna ground, the antenna, and the control site. Red pine is scheduled to be planted in spring 1984. Bud burst and candle elongation will be recorded for red pine. Initial, average, and terminal date of occurrence will be recorded for each event.

Methodologies to be used in describing phenophases were developed during this reporting period. Photographs were taken of each species at various time intervals to document various phenophases. At times these intervals were as short as one or two days. Since a phenophase represents a range of conditions, the photographs attempt to define a particular point in time when that phenophase is said to occur. For instance, bud burst is a continuing process and does not represent a single point in time. The photographs then identify a specific stage of the phase that will be used to document the event.

Photographs were taken during bud swell, bud burst, leaf out, and flowering. The photo sequence developed for trembling aspen is complete. However, trembling aspen was not found on all of the study sites and consequently, will not be monitored further. Bud burst, and leaf out has been documented for paper birch, but more photographs are needed to further document flowering and seed dissemination. Preliminary work has been started on red oak and red maple. These species were not included in the original study plan but are major components of the forest community on the study plots and should be included in the study. Photo documentation will be completed in 1984 after a new control plot is located.

Cambial activity will be monitored by use of permanently installed dendrometer bands. Measurements will be taken weekly starting in early spring 1984 to document the initiation of cambial activity and monthly thereafter to obtain growth and productivity data (see Element 3: Tree Productivity Studies).

Element 5. Herbaceous Vegetation Cover and Growth

The primary objective of this phase of the project is to detect changes in the aboveground productivity of a few selected forest herbs that might result from the introduction of ELF fields. A subsidiary objective is to detect the effect that such fields might have on the relative species composition of forest ground cover plants.

Progress

A preliminary reconnaissance of the plots was made to determine the relative abundance of herbaceous plants prior to plot selection. Voucher specimens were collected and unknowns were identified. Comparing the results of this survey for the antenna plots with those of the control plots, three herb species were found to be the most abundant on both sites. These were Pteridium aquilinum, Trientalis borealis, and Aster macrophyllum.

For each of these three species, ten 1m^2 quadrats were established at each site within areas where colonies of the species could be found. These plots were set up to non-destructively sample shoot dry weights by using regression equations to estimate plant weight from dimension measurements. Measurements included stem length, leaf length (longest dimension of the perimeter of the single horizontal whorl), leaf diameter (measurement of whorl perpendicular to that taken for leaf length), and total height. All dimension measurements were in units of centimeters. By using this non-destructive method, productivity can be compared from year to year on the same quadrats. Studies have determined that regression equations can be developed in estimating plant biomass (Baskerville, 1972).

Regression Analysis

In order to establish the regression equations, the aboveground portions of shoots of each species were harvested outside the quadrats. After the appropriate dimensions were measured, each shoot was oven-dried and weighed. These weights were broken down into stem weight, leaf weight, fruit weight, and flower weight (when the last two were available). These data were then used to develop a regression equation to predict oven dried shoot weight (gm) for each respective species. Measurements from the developmental and validation data used in estimating these regression equations covered a range of dimensions similar to that existing on the quadrats. Careful consideration will be made to use these equations with data within the range for which they were developed.

One hundred Trientalis borealis plants or portion of plants (each stem and attached leaves) were measured and weighed. Total weight (gm) ranged from a high of 0.3620 and a low of 0.0180 with a mean of 0.1232 and a standard deviation of 0.0737. Of the one hundred sampled, 73 of these observations were used for model development and 27 of these observations were used for validation of candidate models. The final model was selected based on the R², MSE, and Cp values, as well as its performance with the validation data. Coefficients reported below for the chosen model were estimated from the complete data set of 100 observations. The resulting model was of the form:

$$\ln Y = b_0 + b_1 \ln S + b_2 \ln A$$

Where:

\ln = natural logarithm

Y = total weight (gm)

S = stem length (cm)

$$A = \frac{(\text{leaf length (cm)})^2 + (\text{leaf diameter (cm)})^2}{2}$$

$$b_0 = -7.0855$$

$$b_1 = 0.1643$$

$$b_2 = 0.9048$$

This equation produced an $R^2 = 0.9194$ and a mean square error (MSE) = 0.0396. Aster macrophyllum had 182 observations i.e. each stem and attached leaves were measured and weighed. The plants on both sites consisted only of basal leaves and to simplify estimating biomass, each leaf was treated as a separate shoot with petiole length S , leaf blade length, and leaf blade width. Samples of total weight (gm) ranged from a high 0.6100 gm to a low of 0.0070 gm with a mean of 0.1343 gm and a standard deviation of 0.1022 gm. Of the total number of observations, 155 observations were randomly selected and used as data for model development; the remaining 27 observations were used to validate the candidate models. The final model was again chosen based on values of R^2 , MSE, and CP as well as the performance of validation data. Coefficients reported below for the model chosen were estimated from the complete data set of 182 observations. The resulting model was of the form:

$$\ln Y = b_0 + b_1 \ln S + b_2 \ln A$$

where:

\ln = natural logarithm

Y = total weight (gm)

S = stem length (cm)

A = leaf blade length (cm) x leaf blade width (cm)

$$b_0 = -5.5082$$

$$b_1 = 0.5958$$

$$b_2 = 0.6284$$

This equation produced an $R^2 = 0.9207$ and a mean square error (MSE) = 0.0573.

As yet we have not developed a satisfactory regression equation for Pteridium aquilinum. If we fail to develop such, we plan to take a more detailed set of measurements next summer.

The dry weights of the plants harvested for the regression development were also used to estimate an average plot mean and variance of shoot dry weights for the selected species and from this determine the bounds within which we can expect to estimate this quantity in the future. No plot data of shoot dry weights or changes in shoot dry weight are available this season. Mean weights and variances of the means from off plot observations were assumed to be comparable to weights of plants on the sites and these values were expanded to calculate an average plot mean and variance per site. These are conservative estimates until actual data can be obtained. Using the variance obtained from these samples, a value of n corresponding to the number of 1m^2 plots taken on a site, and a 95% level of confidence, the bounds or the confidence interval was calculated for each species for both the antenna and control sites. One is 95% confident of detecting a change in biomass within the bounds determined on each 1m^2 plot. For example, on the antenna site, one is 95% confident of detecting a change of 1.39 g on each 1m^2 plot. Table 7 gives the mean (gm), the variance (gm), the total number of 1m^2 plots on the site, and the bounds for detection of a difference for each species on each site:

Table 7. Biomass and bounds summary for herbaceous plants.

Species/site	Average lm^2 plot mean	Average lm^2 plot var.	n	+ Bounds
<u>Aster macrophyllum</u>				
Antenna	2.5551	3.7956	10	1.39
Control	5.3630	28.7631	10	3.83
<u>Trientalis borealis</u>				
Antenna	0.8034	0.2306	10	0.34
Control	1.4585	0.7599	10	0.34
<u>Pteridium aquilinum</u>				
Antenna	23.2657	221.6502	9	11.44
Control	40.0009	675.0061	10	18.58

Due to lack of remeasurement plot data, these results reflect the variability if entirely new samples will be taken each year. Measurements need to be taken several times during the growing season to reflect different growth rates. With remeasurement data available, the variability associated with changes on the same plots from year to year can be calculated and these bounds will be reevaluated and may be reduced. If no remeasurement data can be obtained before the ELF fields are introduced and the number of lm^2 plots are not increased, then these confidence intervals reflect the large change in biomass that must occur before detection of that change could be made at a 95% confidence level with $n-1$ degrees of freedom. Only small increases could be made in the number of quadrats sampled since there are limited numbers of plants on the sites.

After the three species selected for productivity measurements had reached their full development for the season, measurements of appropriate dimensions for all specimens of each of these species inside the lm^2 quadrats were made. Percent cover estimates were also made of all species of low ground cover plants within the quadrats. The latter measurements will be repeated

each year so that changes in species composition can be detected from year to year.

In order to characterize the ground cover flora in the 3 plots at both the antenna and the control sites, percent cover of all ground cover species was estimated by the line-intercept method. For this purpose three 35 m length lines were used within each plot parallel to the long axis of the plot. These measurements will not be repeated annually. From these data Sorenson's index of similarity, as modified by Motyka et al. (1950) for use with quantitative data, was calculated in order to compare the similarity in ground cover between the aboveground and control sites. The formula used is:

$$IS = \frac{2C}{A + B}$$

where IS is the similarity index, C is the lower of any pair of cover values of a species found at both sites, A is the cover value of any species found on one of the two sites, and B is such a value found on the other site.

Also, from the same data, the Shannon index of diversity was calculated for both sites using the formula:

$$H = - \sum_{i=1}^n p_i \ln p_i$$

where n = number of species, and p_i is the proportion of species i to the total (the total percent coverage for each species).

Percent cover values of low ground cover plants as derived from the line-intercept sampling indicates that the antenna site is dominated by Pteridium aquilinum, Rubus parviflorus, Rubus idaeus, and Gaultheria procumbens while the control site is dominated by Pteridium aquilinum, Carex peckii, Gaultheria

procumbens, Aster macrophyllum, and Vaccinium membranaceum. Sorenson's index of similarity for the two sites is 0.58. The Shannon index of diversity for the antenna and control sites are 1.55 and 1.74, respectively. The sample size (number of plots) was the same on each site, thus the Shannon indices for the two sites are comparable. Sorenson's index indicates a moderate level of similarity in species mix between the sites, but Shannon's index indicates a very similar degree of diversity within each site.

Unfortunately, after all of the summer's data was taken, the decision was made to abandon the control site. For this reason calculations of dry weights from the plant dimensions taken in the quadrats were not made. Also, the similarity indices as calculated from the line-intercept data from 1983, will probably be of little use in the future.

Element 6: Mycorrhizal Fungi Collection

Mycorrhizae represent an ideal system with which to detect environmental perturbations in forest ecosystems such as might be caused by ELF electromagnetic fields. The mycorrhiza phenomenon is a balanced physiological relationship between the roots of higher plants and a variety of highly specialized and beneficial parasitic fungi. In return for host carbohydrates, mycorrhizal fungi permeate the forest floor, providing their hosts with minerals and water more efficiently than could the hosts' roots alone.

Two major types of mycorrhizae form on forest trees: ectotrophic and endotrophic mycorrhizae. Ectotrophic mycorrhizae lend themselves to extensive population dynamics study more easily than do endotrophic types. First, ectotrophic fungi produce a mantle which affects feeder root development, resulting in more or less characteristic rootlet morphology patterns. Second, ectotrophic fungi characteristically produce relatively large and identifiable fruiting bodies which aid greatly in population studies. Finally, many ectotrophic fungi may be isolated into pure culture from fruiting bodies or surface-sterilized feeder roots. On the other hand, endotrophic mycorrhizal fungi do not influence rootlet morphology. As a result, infected roots cannot be characterized or distinguished from uninfected roots without time-consuming laboratory preparation and microscopy. Furthermore, endotrophic fungi do not produce macroscopic fruiting bodies and have not been successfully cultured on artificial media to date. For the above reasons, the mycorrhizae studies represented by work elements 6 and 7 are restricted to ectotrophic mycorrhizal fungi and their hosts.

Establishment of a reference collection containing cultures and/or freeze-dried fruiting bodies of identified mycorrhizal fungi serves two purposes. First, mycorrhizal fungi isolated into pure culture from roots can only be identified by comparison of their cultural growth characteristics with those of known species isolated into pure culture from identified fruiting bodies. Second, the reference collection of identified fruiting bodies broadens our perspective concerning mycorrhizal fungus populations in the study area by including mycorrhizal fungi which cannot be isolated into pure culture on known media as well as fungi associated with tree species not targeted for intensive root study (see Element 7). Periodic monitoring of fruiting over an extended period is necessary because mycorrhizal fruiting varies phenologically and is somewhat ephemeral. Ambient monitoring data will be used to account for the influences of moisture and temperature on fruiting over time at the different study sites. Techniques for characterizing and comparing fungal populations via fruiting body production have been published (Grainger 1946, Parker-Rhodes 1951, Hering 1966, Richardson 1970, Fogel 1976, Fogel 1981). Similarities in overstory species composition of ectomycorrhizal hosts and basic soils properties between the antenna pole-stand site and the most-likely-to-be-selected control pole-stand site support the presumption that mycorrhizal populations at the two study sites should be well matched. The quantitative techniques to be used, however, do not require that sites be perfectly matched initially in order to detect relative population changes which develop over time experimentally. Any effects of ELF fields on the fruiting dynamics of mycorrhizal fungi will be detected as shifts in the relative representation of fruiting species at each site over a period of years.

Progress

Fruiting bodies of ectomycorrhizal fungi occurring on the 30 m x 35 m study plots were inventoried at irregular intervals between late August and early October, 1983. Whenever possible, sufficient specimens were collected for freeze-dry preservation and isolation attempts. Although field notes include the closest ectomycorrhizal tree species, the mixed species composition of the study stands and the uncertain breadth of host range for these fungi preclude establishment of firm relationships.

All isolation attempts from fruiting bodies were made by planting small portions of stipe or pileus context tissue onto modified Hagem agar medium (Molina and Palmer 1982), the same medium used for isolation attempts from mycorrhizal root tips. Between 10 and 20 isolations were attempted from each selected specimen. Table 8 presents the results of isolation attempts from 28 reportedly ectomycorrhizal fungi. Table 9 presents a listing of reportedly ectomycorrhizal species identified from the study sites and represented in the collection of freeze-dried specimens. At least 5 additional species were recorded infrequently on study sites.

It became apparent during the autumn of 1983 that mycorrhizal fungus populations should continue to be monitored on study plots via fruiting body production. This method has the advantages of including fungal species which cannot be isolated into pure culture from roots as well as fungal species associated with tree species other than red pine. Data collected in 1983 suggest that sufficient fruiting by a number of mycorrhizal species does take place to permit detection of any meaningful population disturbance.

A systematic method of periodic monitoring will be adopted for the 1984 field season in order to facilitate evaluation of fruiting dynamics for

Table 8. Results of Isolations Attempted From Fruiting Bodies of Suspected Mycorrhizal Fungi Occurring on Study Sites

Family	Genus	Species	Result ^A
Amanitaceae	<u>Amanita</u>	<u>bisporigera</u> Atk.	
		<u>brunnescens</u> Atk.	+
		<u>citrina</u> (Schaeff. ex) S.F. Gray	+
		<u>muscaria</u> (L. ex Fr.) Pers. ex Hooker	+b
		<u>vaginata</u> (Bull. ex Fr.) Vitt.	
Boletaceae	<u>Boletus</u>	<u>piperatus</u> Bull. ex Fr.	+b
		<u>Leccinum</u>	
		<u>insigne</u> Smith, Thiers, & Watling	+b
		<u>scabrum</u> (Bull. ex Fr.) S.F. Gray	+b
		<u>subglabripes</u> (Peck) Sing.	+
	<u>Suillus</u>		
		<u>granulatus</u> (L. ex Fr.) Ø. Kuntze	+
Cantharellaceae	<u>Cantharellus</u>	<u>lutescens</u> Fr.	
Cortinariaceae	<u>Cortinarius</u>	<u>flavifolius</u> Peck	
		undetermined "B"	
	<u>Hebeloma</u>	<u>crustuliniforme</u> (Bull. ex St. Am.)	Quel.+
		<u>mesophaeum</u> (Pers.) Quel.	
	undetermined "A"	+	
	<u>Rozites</u>	<u>caperata</u> (Pers. ex Fr.) Karst.	
Paxillaceae	<u>Hygrophoropsis</u>	<u>aurantiaca</u> (Wulfen ex. Fr.) R. Maire	+
Russulaceae	<u>Lactarius</u>	<u>argillaceifolius</u> Hesler & Smith	+
		<u>piperatus</u> (L. ex Fr.) S.F. Gray	
		<u>subvellereus</u> Peck var. <u>subdistans</u> Hesler & Smith	+
		<u>vinaceorufescens</u> Smith	+
	<u>Russula</u>	<u>aeruginea</u> Lindblad apud Fr.	
		<u>variata</u> Bann. apud Peck	
Tricholomataceae	<u>Clitocybe</u>	<u>gibba</u> (Pers. ex Fr.) Kummer	+
		<u>odora</u> (Bull. ex Fr.) Kummer	+
	<u>Laccaria</u>	<u>laccata</u> (Scop. ex Fr.) Berk. & Br.	+
		<u>Tricholoma</u>	<u>flavovirens</u> (Pers. ex Fr.) Lundell
	apud Lund. & Nannf.		+

a. Successfully isolated into pure culture (+); unsuccessful (-).

b. Occurring on study site, but isolated from other collections.

Table 9. Species of Presumably Mycorrhizal Fungi Currently Represented in the Freeze-dried Specimen Collection

Family	Genus	Species
Amanitaceae	<u>Amanita</u>	<u>bisporigera</u> Atk. <u>brunnescens</u> Atk. <u>citrina</u> (Schaeff ex) S.F. Gray
Boletaceae	<u>Boletus</u>	<u>vaginata</u> (Bull.ex Fr.) Vitt. <u>piperatus</u> Bull.ex Fr. undetermined "A"
	<u>Leccinum</u>	<u>insigne</u> Smith, Thiers & Watling <u>scabrum</u> (Bull.ex Fr.) S.F. Gray
	<u>Suillus</u>	<u>granulatus</u> (L. ex Fr.) Ø. Kuntze
Cantharellaceae	<u>Cantharellus</u>	<u>lutescens</u> Fr.
Cortinariaceae	<u>Cortinarius</u>	<u>armillatus</u> (Fr. ex Fr.) Fr. <u>callisteus</u> (Fr. ex Fr.) Fr.a <u>caninus</u> (Fr.) Fr.a <u>cinnamomeus</u> Fr.a <u>collinitus</u> (Sow.ex Fr.) Fr.a <u>flavifolius</u> Pecka <u>olivaceus</u> Pecka <u>sanguineus</u> (Fr.) Fr. <u>semisanguineus</u> (Fr.) Gill. undetermined "A" undetermined "B"
	<u>Hebeloma</u>	<u>crustuliniforme</u> (Bull.ex St. Am.) Quel) <u>mesophaeum</u> (Pers.) Quel. <u>caperata</u> (Pers. ex Fr.) Karst. undetermined "A"
Elaphomycetaceae	<u>Rozites</u>	
Gomphidiaceae	<u>Elaphomyces</u>	
Hydnaceae	<u>Chroogomphus</u>	<u>vinicolor</u> (Peck) Ø.K. Miller
	<u>Hydnellum</u>	<u>zonatum</u> (Fr.) Karsten
	<u>Hydnum</u>	<u>imbricatum</u> Fr.
Hygrophoraceae	<u>Camarophyllus</u>	<u>borealis</u> (Peck) Murr.
Paxillaceae	<u>Hygrophoropsis</u>	<u>aurantiaca</u> (Wulfen ex. Fr.) R.
Maire		
Rhodophyllaceae	<u>Rhodophyllus</u>	<u>lividus</u> (Bull. ex Merat) Quel.

Table 9. Con't.

Family	Genus	Species
Russulaceae	<u>Lactarius</u>	<u>argillaceifolius</u> Hesler & Smith <u>chrysorheus</u> Fr. <u>fumosus</u> Peck <u>subvellereus</u> Peck var. <u>subdistans</u> Hesler & Smith <u>vinaceorufescens</u> Smith
	<u>Russula</u>	<u>aeruginea</u> Lindblad apud Fr. <u>albida</u> Pecka <u>albonigra</u> (Krumbh) Fr. <u>amygdaloides</u> Kauffm. ^A <u>brevipes</u> Peck <u>emetica</u> (Schaff. ex Fr.) Pers. ex Fr. <u>fragilis</u> (Pers. ex Fr.) Fr. ^A <u>laurocerasi</u> Melzer <u>paludosa</u> Britz. <u>rubescens</u> Beardsleea <u>squalida</u> Pecka <u>tenuiceps</u> Kauffm. ^A <u>xerampelina</u> (Schaeff. ex Secr.) Fr. <u>variata</u> Bann. apud Peck
Tricholomataceae	<u>Clitocybe</u>	<u>gibba</u> (Pers. ex Fr.) Kummer <u>odora</u> (Bull. ex Fr.) Kummer
	<u>Laccaria</u>	<u>laccata</u> (Scop. Ex Fr.) Berk. & Br.
	<u>Leucopaxillus</u>	undetermined "A"
	<u>Tricholoma</u>	<u>flavovirens</u> (Pers. ex Fr.) Lundell apud Lund. & Nannf.

^A Specific epithet as yet uncertain.

comparisons between plots and over time. Quantitative concepts useful for comparing data sets include major species (Hering 1966), middate of fruiting (Richardson 1970), coefficient of community (Pielou 1977), and Orloci's sums of squares method based on standardized distances (Orloci 1967).

Element 7: Mycorrhiza Characterization and Root Growth

Introduction

For the purposes of the environmental monitoring program, we initially proposed to study the ectotrophic mycorrhizae formed with balsam fir and paper birch. Both tree species were expected to be abundant at study sites, and both species are characterized as predominantly ectomycorrhizal with relatively few associated species of mycorrhizal fungi.

Red pine has finally been chosen for intensive root study instead of balsam fir and paper birch for the following reasons. Once the ELF antenna ground locations were selected, it became apparent that few balsam fir would be present on study plots, and that paper birch would be represented by scattered pole and sawtimber-sized trees. Because balsam fir is a difficult species to establish by planting, it was decided to work with red pine instead. Red pine is likewise scattered naturally throughout the study area, and has several advantages for study over balsam fir. First, red pine is easier to establish by planting, and red pine planting stock is much more readily available. Second, more is known about red pine mycorrhizae than about those of balsam fir. Third, red pine lends itself better to study of seedling growth and vigor due to its excurrent growth habit.

Paper birch study has been discontinued for three major reasons. First, paper birch mycorrhizae were found to be extremely fine structures, densely packed and interwoven with mycelium. The logistics of unraveling birch mycorrhizae for quantitative study proved unfeasible. Second, the abundance of beaked hazelnut (also in the birch family, Betulaceae) on study plots necessitated excavation of birch roots in order to be certain that birch rather than hazelnut roots were collected. Excavation of birch roots proved

to be both time-consuming and disruptive to the study plots. Finally, establishment of paper birch seedlings for study would be difficult. The effort originally budgeted for paper birch study will be effectively utilized to enhance studies of 1) red pine mycorrhizae and 2) the population dynamics of fruiting by ectotrophic mycorrhizal fungi (Element 6).

To date, mature red pine on or near study plots and seedlings from the Toumey National Forest Nursery in Watersmeet, Michigan, have been sampled by soil core collection and seedling excavation, respectively. As soon as the necessary small-scale clearcuts are made during the spring of 1984, red pine study plantations will be created 1) at the buried antenna study site, 2) across the antenna right-of-way from the antenna pole-stand study site, and 3) across the sham right-of-way from the control pole-stand study site. The plantation sites offer abundant inoculum of fungi capable of forming ectomycorrhizae with red pine. Because plantations will be established immediately after clearing, the mycorrhizal complement of the original stand will remain viable long enough to support red pine seedling establishment to the extent that it is capable. In addition, the small size of the clearcuts will ensure abundant airborne inoculum from surrounding stands.

Despite the changes outlined above, the basic experimental design for data analysis remains the same as originally documented.

Progress

As field reconnaissance of potential study sites progressed, it became clear that balsam fir would not be satisfactorily represented and would have to be replaced by red pine as the conifer study species. It also became clear that numbers of red pine and paper birch seedlings occurring in the understory were insufficient to permit excavation of natural seedlings as the standard mycorrhizal sampling technique. Sufficient pole-size paper birch occurred, however, to offer hope that soil coring or partial root excavation would provide satisfactory mycorrhizal samples. It was also recognized that red pine would have to be planted on study sites, but that initial study could be effectively conducted utilizing mature red pine growing at the study sites. Because planting will be necessary, study of red pine nursery stock was initiated by seedling excavation at the Toumey National Forest Nursery in Watersmeet, Michigan.

The first task at hand was to test soil coring as a technique for obtaining mycorrhizal root samples from overstory birch and pine study trees on the study plots. The aid of a wood technologist was enlisted in order to identify mycorrhizal roots sampled to species on the basis of root anatomy. Pine presented no problems due to the characteristic presence of resin ducts. On the other hand, no reliable means is available for rapid separation of paper birch roots from beaked hazelnut roots. Initial study concluded that 1) study of paper birch would necessitate excavation of mycorrhizal roots by following a lateral root into the soil for positive identification of sample material, and 2) excavation of red pine mycorrhizae by the same method is a more productive and efficient technique than soil coring.

Selection of trees for sampling at study sites involved selection of healthy individuals, recognizing that vigorous trees would produce more roots and best represent the mycorrhizal fungus population at the given site. Three red pine and three paper birch trees were selected for study at the and control study sites. Three red pine study trees were selected at each endpoint plantation site.

Continuity of sampling has provided useful information for the antenna study site. However, relocation of the antenna ground site was necessitated by alteration of the antenna endpoint/ground configuration during the summer of 1983. Also, relocation of the control study site has been necessitated twice. Both control sites were rejected by IITRI on the basis of background electromagnetic fields present. An approved control site remains to be located.

On June 22, 1983, the study sites were sampled for mycorrhizae using the soil core method. Ten cm diameter soil cores were taken through the litter, organic and A horizons at the canopy margin of each study tree. Four cores per tree were taken representing the cardinal directions. Cores were bagged and refrigerated prior to laboratory analysis. Soil and extraneous organic matter was removed from cores as follows. Cores were washed in approximately 20 to 30 changes of running tap water while gently manipulating roots to loosen and remove all extraneous material. A final washing was conducted in an ultra-sonic bath with a few drops of surfactant (Tween 80) for one to two minutes. The root mat was then placed in a pan of water and the study tree roots were separated by hand. As a result of this first sampling, seven morphology types were identified on paper birch and six on red pine.

On July 19, 1983, mycorrhizae were again sampled on the ELF study sites. Instead of soil coring, feeder roots bearing mycorrhizae were excavated by hand by following main roots out from the base of the study trees. A trowel was used to remove feeder roots, carefully keeping enough surrounding soil intact to minimize damage to, and loss of, attached mycorrhizae. This method supplies more mycorrhizae and eliminates confusion between birch and hazel roots. Roots collected in this manner required less washing than soil cores, but otherwise mycorrhizae were cleaned to the same degree as before. Three additional morphology types were observed on paper birch and two on red pine.

On October 10, 1983, mycorrhizae were again sampled by the hand excavation method. One additional morphology type was observed on red pine. Paper birch was sampled but not treated in the laboratory due to the difficulties mentioned above. The occurrence and distribution of mycorrhizal morphology type categories present on paper birch and red pine at the ELF study sites are summarized in Table 10.

Since ample feeder roots bearing mycorrhizae were collected from red pine by hand excavation, individual mycorrhizae were counted to quantify the frequency of occurrence of morphology type categories. A dissecting microscope was used to identify and tabulate the individual mycorrhizal tips. The results are shown in Table 11.

Ten individual tips of each mycorrhizal morphology type category were surface-sterilized and incubated on Hagem agar, a standard mycorrhizal fungus growth medium, to isolate possibly mycorrhizal fungi. The numbers of fungi isolated from the morphology types is shown in Table 12.

All mycorrhizal and non mycorrhizal fungi isolated are being refrigerated in sterile water for long term storage. They will be revived as time permits for further study, characterization and possible identification to species.

Table 10. Mycorrhizal morphology types present on paper birch and red pine study trees on the ELP study sites.

Study Tree and Site	Morphology Types Present by Sampling Data		
	22 June 1983	19 July 1983	10 October 1983
<u>First Endpoint</u>			
Pine No. 1	1, 2, 4	1, 2, 4, 7, 9	—
Pine No. 2	—	1, 2, 4	—
Pine No. 3	—	1, 2, 4, 7, 8, 9	—
<u>Antenna</u>			
Pine No. 4	2, 6	1, 2, 4, 6, 7	1, 2, 3, 7
Pine No. 5	1, 2, 6, 7	1, 2, 4, 6, 7, 9	1, 2, 7
Pine No. 6	1, 2, 7	1, 2, 4, 6, 7, 8, 9	1, 2, 7, 10
Birch No. 2	1, 2, 3	1, 2, 3, 5, 6, 7	—
Birch No. 3	1, 2, 3	1, 2, 3, 4, 5, 6	—
Birch No. 4	1, 2, 3, 4	1, 2, 3, 5, 6, 7	—
<u>First Control</u>			
Pine No. 7	1, 2, 4, 6, 8	—	—
Pine No. 8	1, 2, 4, 6, 7, 8	—	—
Pine No. 9	1, 2, 4, 6, 7, 8	—	—
Birch No. 5	1, 2, 3, 5, 6	—	—
Birch No. 6	1, 2, 5, 6, 7	—	—
Birch No. 7	1, 2, 5, 6, 7	—	—
<u>Second Endpoint</u>			
Pine No. 1a	—	—	1, 2, 7
Pine No. 2a	—	—	1, 2, 6, 7, 9
Pine No. 3a	—	—	1, 2, 6, 7
<u>Second Control</u>			
Pine No. 10	—	1, 2, 4, 6, 7, 10	—
Pine No. 11	—	1, 2, 4, 7, 8, 9, 10	1, 2, 6, 7, 8
Pine No. 12	—	1, 2, 4	1, 2
Pine No. 13	—	—	1, 2, 6
Birch No. 8	—	1, 2, 3, 5, 6, 7, 8	—
Birch No. 9	—	1, 2, 3, 5, 6, 7, 8, 9	—
Birch No. 10	—	1, 2, 5, 6, 7, 9	—

Table 11. Occurrence and distribution of mycorrhizal morphology types on red pine feeder roots at the ELF study sites.

Date	Study Tree	Feeder Root Length Examined (cm)	Numbers of Mycorrhizae Observed by Morphology Type									
			1	2	3	4	5	6	7	8	9	10
19 July 1983	Pine #1	100.6	92	149	-	11	-	-	46	-	4	-
10 Oct. 1983	Pine #1a	128.5	177	97	-	-	-	-	9	-	-	-
19 July 1983	Pine #2	105.5	130	141	-	44	-	-	-	-	-	-
10 Oct. 1983	Pine #2a	44.5	71	35	-	-	-	19	9	-	1	-
19 July 1983	Pine #3	107.5	406	171	-	1	-	-	28	2	34	-
10 Oct. 1983	Pine #3a	69.0	123	116	-	-	-	2	4	-	-	-
19 July 1983	Pine #4	100.0	395	71	-	7	-	8	7	-	-	-
10 Oct. 1983	Pine #4	58.0	59	47	57	-	-	-	3	-	-	-
19 July 1983	Pine #5	100.0	306	62	-	36	-	13	13	-	3	-
10 Oct. 1983	Pine #5	82.5	82	13	-	-	-	-	51	-	-	-
19 July 1983	Pine #6	100.0	179	53	-	6	-	1	6	6	7	-
10 Oct. 1983	Pine #6	45.0	61	76	-	-	-	-	22	-	-	3
19 July 1983	Pine #10	100.0	174	60	-	8	-	25	1	-	-	6
19 July 1983	Pine #11	100.0	217	99	-	4	-	-	2	11	23	16
10 Oct. 1983	Pine #11	86.5	141	15	-	-	-	104	14	10	-	-
19 July 1983	Pine #12	100.0	334	20	-	23	-	-	-	-	-	-
19 Oct. 1983	Pine #12	31.5	51	18	-	-	-	-	-	-	-	-
19 Oct. 1983	Pine #13	62.0	127	54	-	-	-	1	-	-	-	-

Table 12. Numbers of possibly mycorrhizal fungus species isolated on Hagem's agar from surface-sterilized paper birch and red pine mycorrhiza morphology type categories.

Mycorrhiza Morphology Tree Category	Numbers of Fungal Species Isolated	
	22 June and 19 July	10 October 1983
Pine 1	19	5
Pine 2	13	17
Pine 3	--	6
Pine 4	27	--
Pine 6	7	5
Pine 7	11	10
Pine 8	9	--
Pine 9	8	--
Pine 10	5	--
Birch 1	10	--
Birch 2	9	--
Birch 3	10	--
Birch 4	--	--
Birch 5	23	--
Birch 6	5	--
Birch 7	13	--
Birch 8	4	--
Birch 9	6	--

By these means, the patterns of occurrence, distributions, and relationships of these fungi to their plant associates will be defined.

Seedlings from the J. W. Toumey Nursery, Watersmeet, Michigan, will be planted on the ELF study sites plantation plots in order to monitor mycorrhizal fungus population changes over time. Baseline data will consist of the mycorrhizal condition of 3-0 red pine planting stock from the nursery beds. Mycorrhizae morphology type categories and populations of associated mycorrhizal fungi will be evaluated.

On July 8, 1983, 3-0 red pine seedlings in nursery beds of a seed source which would regularly be planted in the Upper Peninsula were excavated by hand. Seedlings were selected from three widely separated locations in three different nursery beds designated A, B and C. Soil was retained with the roots to minimize damage to the attached mycorrhizae. Root systems were washed in the laboratory and studied to determine the morphology types present. Four categories occurred and were designated T1, T2, T3 and T4. These four categories were unique to the Toumey Nursery.

Ten cm sections of feeder root bearing mycorrhizae were examined using a dissecting microscope and the morphology types counted along their length. Thirty sections of 10 cm were examined from each bed, or a total of 300 cm feeder root per bed. The occurrence of mycorrhizal morphology types in each of the seedling beds sampled is shown in Table 13.

Thirty individual tips of each mycorrhizal morphology type were surface-sterilized and cultured on Hagem's agar, a standard mycorrhizal fungus growth medium. Fourteen fungi differing in growth form were isolated. Their origin and distribution among the morphology type categories is shown in Table 14.

Table 13. Frequency of mycorrhiza morphology type category occurrence in three different beds of 3-0 red pine seedlings (Ottawa National Forest seed source) at the J. W. Toumey Nursery, Watersmeet, Michigan, in July, 1983. 300 cm of feeder root were examined from each bed.

Morphology Type Category	Numbers of Mycorrhizae Observed		
	Bed A	Bed B	Bed C
T1	1669	1451	1413
T2	288	220	180
T3	463	558	532
T4	0	111	89
TOTAL	2420	2340	2214

Table 14. Fungi isolated on to Hagem agar from surface-sterilized mycorrhizae of 3-Ø red pine seedlings lifted in July, 1983, at the J. W. Toumey Nursery, Watersmeet, Michigan.

Fungus Species Designation	Mycorrhizael Morphology Type Category			
	1	2	3	4
T01	3			
T02	3	1	1	
T03	1			
T04	6	12	6	
T05		1		
T06			1	
T07	1			
T08	1	1	1	7
T09		1		
T10		1		
T11	1			1
T12		1	1	
T13		1	1	
T14		1		

One publication has resulted from these studies to date, entitled "Actinomycetes associated with red pine mycorrhizae in the field versus nursery stock". This paper was presented at the Third International Symposium on Microbial Ecology, August 7-12, 1983, held in East Lansing, Michigan. A copy of the abstract is included as Appendix J.

Element 8. Litter Production

Litter production is important in the transfer of nutrients and energy within a vegetative community and is dependent on plant growth processes and environmental conditions such as elevation, soil characteristics, slope, soil water content and weather. The sensitivity of litter production to both internal and external conditions make it a good indicator of possible ELF field effects on trees. Since litter samples can be gathered at frequent intervals, they not only provide an estimate of changes in forest production, but also give an insight on the time of tree component loss such as leaves, bud scales, seeds, woody material, and flower parts. This measurement also serves to verify on a stand basis many of the phenological monitoring measurements that will be taken on individual trees. Additionally, leaf samples taken for nutrient analysis would monitor nutrient accumulation during the growing season and subsequent translocation from the foliage to the branches prior to leaf fall. This physiological process is also sensitive to environmental stress and would be a potential indicator of ELF field effects.

Progress

Litter traps are being used to monitor tree litter production on all sites. Five 1x1 meter litter traps were randomly placed on each permanent measuring plot at the antenna and the control sites in August 1983. Litter in the traps was collected at weekly intervals after the onset of leaf fall in early September.

Prior to leaf fall in late September-early October, foliage was obtained from nine dominant or codominant northern red oak, bigtooth aspen, paper birch and red maple at each site. Samples were collected using a 12 gauge shotgun with a full choke. Various pole samplers with and without ladders and smaller

caliber firearms proved to be too cumbersome or inefficient for foliage collection. Consequently, sampling foliage from the lower, middle and upper crown position, as originally proposed had to be abandoned. All samples were taken from trees off of the permanent measurement plots to minimize site disturbance.

Litter and foliage samples were dried at 60°C in a forced draft oven. The litter was separated into the following categories: (1) leaves, (2) wood, (3) miscellaneous and weighed. All samples were ground to pass a 40 mesh sieve and analyzed for N by Kjeldahl digestion, P by the vanadomolybdate method, and Ca, Mg and K by atomic absorption.

Litter fall on the study plots was much later than normal due to the exceptionally warm summer in 1983. Only small amounts of litter had fallen by the beginning of October (Figure 4). Weekly litter fall rates were significantly different ($P < 0.05$) between the antenna site and the control site with the trees on the control retaining their leaves longer. A significant difference in average leaf litter fall over the two month collection period was also found between the sites ($P < 0.05$) (Table 15). Woody and miscellaneous tree component parts were a small part of the total litter fall and only slight differences between sites were found ($P < 0.05$). The error estimate of the mean for average litter fall was 3.6 g/m² for the antenna site and 3.5 g/m² for the control site, an error percent of 13.8 and 15.6 percent of the mean at 0.05, respectively. One is 95% confident of detecting a difference of 0.5 g/m² on the antenna site and 0.55 g/m² on the control site. This error value is well within acceptable variability standards for litter production studies. An additional four litter traps per plot (40 traps/site) would be required to give litter fall estimates within 10% of the mean. This increase in material supplies cost and labor effort is not warranted for such a small reduction in error estimate.

CUMULATIVE LITTER PRODUCTION

Figure 4.

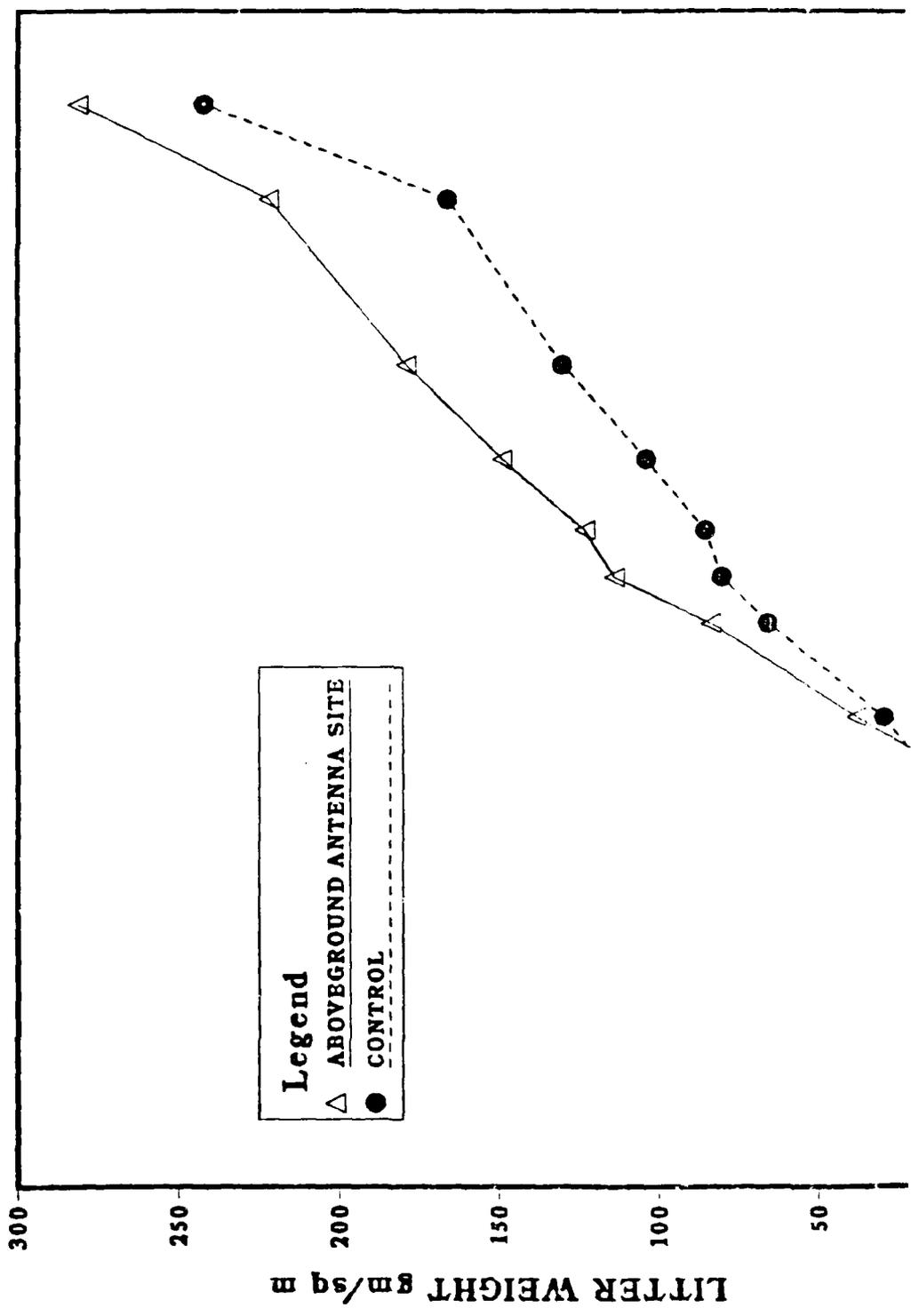


Table 15. Average and Total Litter Fall at the Antenna and Control Sites

Site	Average Litter Wgt/Trap			Total Litter Fall		
	Leaves (gm/m ²)	Wood	Misc.	Leaves (gm/m ²)	Wood	Misc.
Antenna	24.9	0.8	0.4	264.1	7.9	3.5
Control	21.1	0.9	0.3	228.3	10.1	3.2

Foliar nutrient concentrations showed considerable variability among tree species, but little difference was found between sites (Table 16). The nitrogen level in bigtooth aspen was significantly higher in trees growing on the antenna site than on the control. Such a difference in nitrogen concentration is most likely related to a variability among aspen clones rather than to differences in soil nutrient uptake at each site. The other three species sampled did not show a similar pattern. Power of tests were calculated for each nutrient in each species at each site (Table 17). One is 95% confident of detecting a minimum change (as a percent of the mean).

The differences found in litter fall rate and total litter weight likely reflect variations in climatic conditions and stand composition between the antenna site and the control. While it is desirable not to have such variability in litter fall between the two study sites, these results can be accounted for in determining possible ELF field effects by the statistical design incorporated in the overall study. However, these differences have become moot since a new control site will be established this coming year.

Table 16. Foliar nutrient concentrations of four tree species at the antenna and control sites. Different subscripts denote significant difference at $\alpha = 0.05$.

Ca					
	Red Maple	Paper Birch	N. Red Oak	Big Tooth Aspen	Ave.
Antenna	1.20 ^A	1.80 ^A	1.39 ^{AB}	1.63 ^{AB}	1.51 ^X
Control	<u>1.40^{AB}</u>	<u>1.50^{AB}</u>	<u>1.13^A</u>	<u>1.87^B</u>	<u>1.48^X</u>
AVERAGE	1.30 ^{JK}	1.65 ^{JK}	1.26 ^J	1.75 ^K	1.49
Mg					
Antenna	0.26 ^{AB}	0.43 ^C	0.23 ^A	0.41 ^C	0.33 ^X
Control	<u>0.24^A</u>	<u>0.45^C</u>	<u>0.21^A</u>	<u>0.36^{BC}</u>	<u>0.32^X</u>
AVERAGE	0.25 ^J	0.44 ^K	0.22 ^J	0.39 ^K	0.33
P					
Antenna	0.24144 ^A	0.23833 ^A	0.18833 ^A	0.21756 ^A	0.22 ^X
Control	<u>0.23111^A</u>	<u>0.26378^B</u>	<u>0.21622^A</u>	<u>0.21633^A</u>	<u>0.23^X</u>
AVERAGE	0.236275 ^{JKJ}	0.251055 ^K	0.202275 ^J	0.216945 ^{JK}	0.23
K					
Antenna	0.63 ^{AB}	1.06 ^C	1.03 ^{BC}	0.83 ^{ABC}	0.89 ^K
Control	<u>0.49^K</u>	<u>0.92^{BC}</u>	<u>0.82^{ABC}</u>	<u>1.06^C</u>	<u>0.82^X</u>
AVERAGE	0.56 ^J	0.99 ^K	0.93 ^{JK}	0.95 ^{JK}	0.85
N					
Antenna	1.43	1.85	1.81	2.43	1.88 ^X
Control	<u>1.78</u>	<u>1.74</u>	<u>1.92</u>	<u>1.58</u>	<u>1.63^Y</u>
AVERAGE	1.36 ^A	1.80 ^{AB}	1.87 ^B	2.01 ^B	1.76

Table 17. Minimum difference as a percent of the mean that can be detected at $\alpha = 0.05$ level.

Species	Ca		Mj		P		K		N	
	Above Ground	Control								
Red Maple	23%	35%	25%	29%	9%	32%	29%	22%	26%	21%
Paper Birch	26%	25%	24%	19%	11%	15%	28%	27%	8%	17%
N. Red Oak	15%	19%	15%	14%	23%	12%	44%	11%	25%	23%
Big Tooth Aspen	21%	18%	32%	14%	11%	23%	23%	41%	12%	21%

Element 9: Data Management**Progress**

Work conducted under each element of the Trees and Herbaceous Plants Task described previously will generate various quantities of data. These data sets can be relatively small, such as soil profile descriptions or extremely large as in the case of the ambient monitoring data. In many cases it will be necessary to integrate ambient monitoring data with biological data in the statistical analysis in order to separate environmental factors from possible influences due to ELF electromagnetic fields. It became evident that an effective method of handling and manipulating vast quantities of data was needed. Two options were immediately evident: 1) write a set of specialized computer programs, or 2) utilize a commercially available data management system. A large work effort would be required to develop and debug such sophisticated computer programs. In addition, these programs possess the flexibility to accommodate unforeseen changes in future data collection.

Cost analysis showed that writing, debugging, maintaining, and updating data management programs would be more costly throughout the remainder of this project than purchasing an existing data management system. In addition, the Academic Computing Services at Michigan Tech will support and maintain a commercial system on the University's UNIVAC 1100/80 computer. Based on this evaluation, we decided to investigate available data management systems and found that SIR DBMS (Scientific Information Retrieval Database Management System) appeared best suited to the needs of the project. The package was obtained for a 90 day trial evaluation, which included an introductory training course. Evaluation of SIR DBMS indicated that it would perform all

data management functions necessary for the statistical analysis required by the Trees and Herbaceous Plants Task. The 90 day evaluation concluded at the end of this reporting period with purchase of SIR DBMS dependent on funding levels in FY 1984.

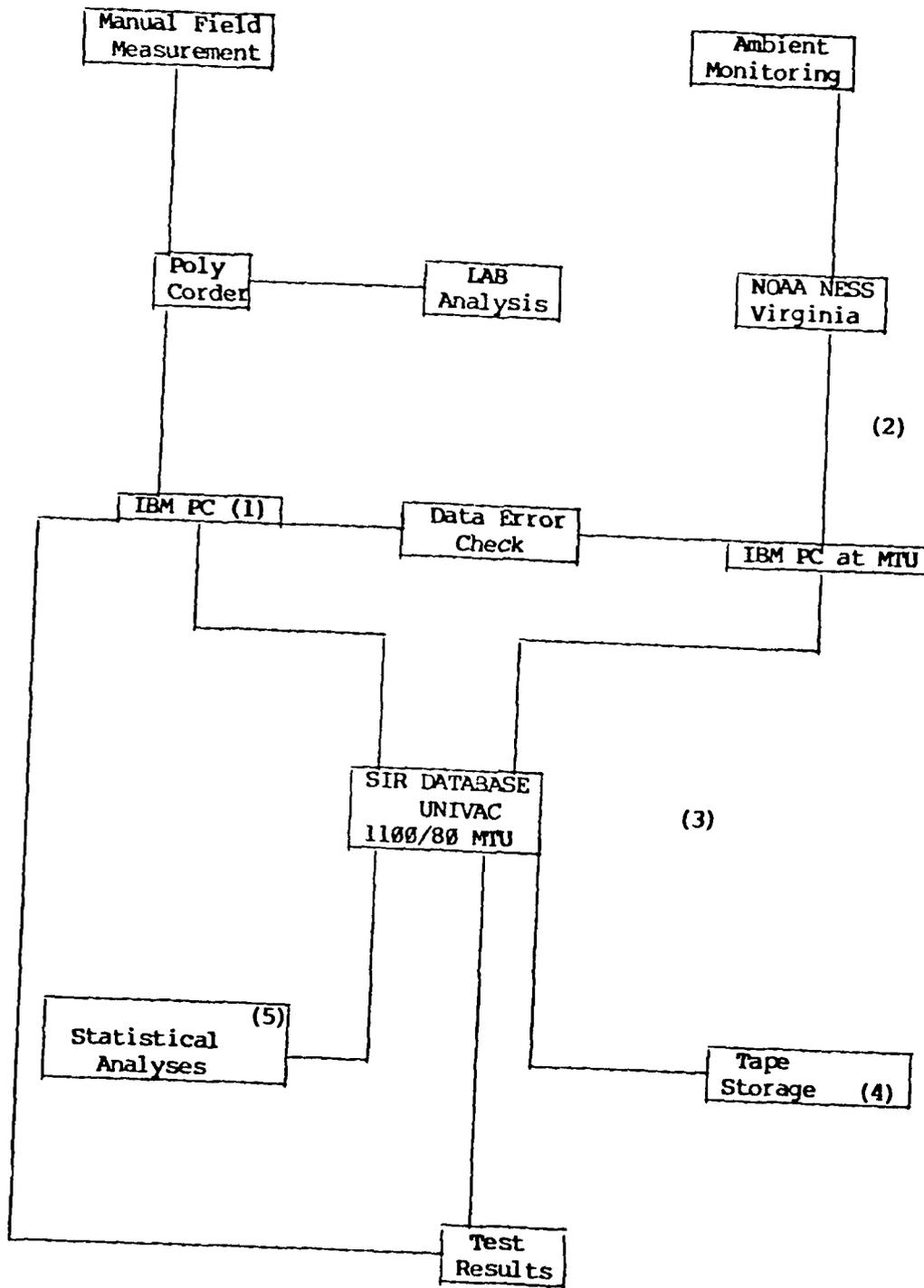
Meetings were held with each principal investigator to determine the type, amount and frequency of data each was collecting. Based on this information, an initial database structure was developed and is presented in Appendix H. Data form design and methods of data input were also discussed so that data formats were standardized and compatible with SIR DBMS.

The general data management plan for both the Trees and Herbaceous Plants Litter Decomposition Tasks is shown in Figure 5. The field measurements will be entered on a Polycorder (an electronic data logging device), or samples collected and tagged for further analysis. Data entered on the Polycorder will be transferred to the onsite IBM Personal Computer (1) for error checking. Laboratory analysis data will also be entered and error checked on the IBM PC as it becomes available. Data will then be transferred from the on site IBM PC to the UNIVAC 1100/80 SIR Database using a 300 baud asynchronous dialup line.

The daily ambient monitoring information will be transferred via satellite to Virginia (NESS) for initial storage (refer to Element 2). A 300 baud asynchronous dialup phone line will be used to "talk" to NESS daily and transfer the data to an IBM PC at Michigan Technological University (2, Figure 5). The IBM PC will then transfer data using a 1200 baud asynchronous dialup line to the UNIVAC 1100/80 SIR Database.

The UNIVAC 1100/80 SIR Database (3) will then be used to store, retrieve and aid in the analysis of both the ambient monitoring and field information. Data will be intermittently written to tape (4) for backup. In addition, data not currently being analyzed will be unloaded from the database and placed on tape to reduce file storage costs.

Figure 5. DATA MANAGEMENT FLOWCHART



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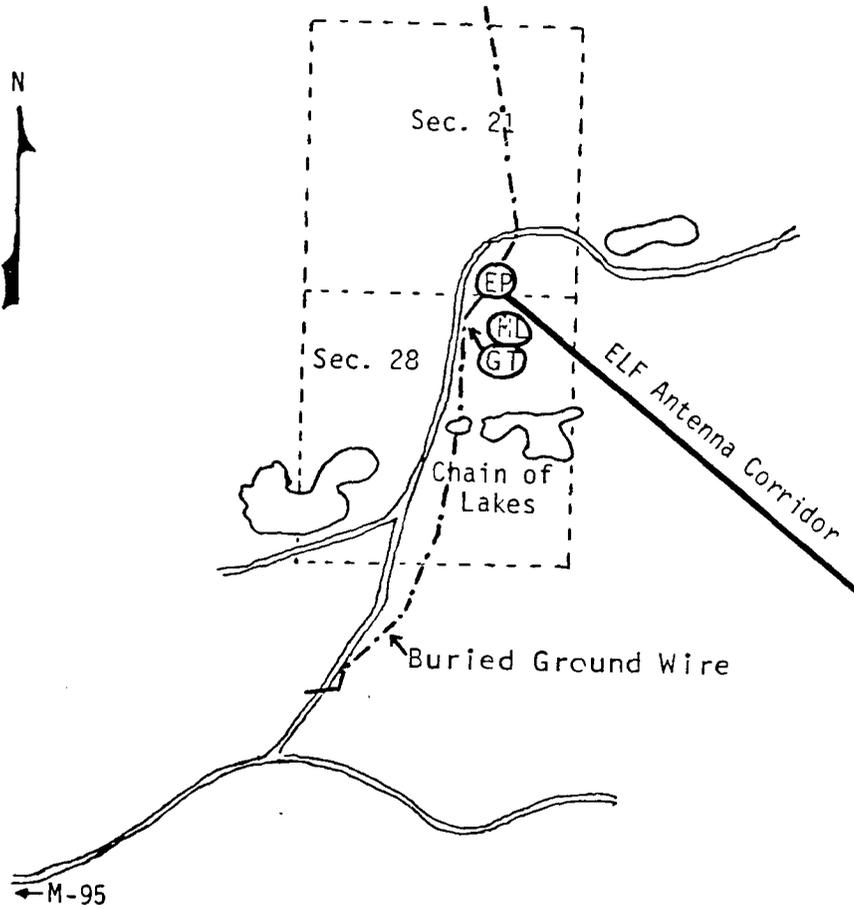
APPENDIX A

Plot location maps for the trees and herbaceous plant cover task

ELF ANTENNA PLOTS

T45N, R29W

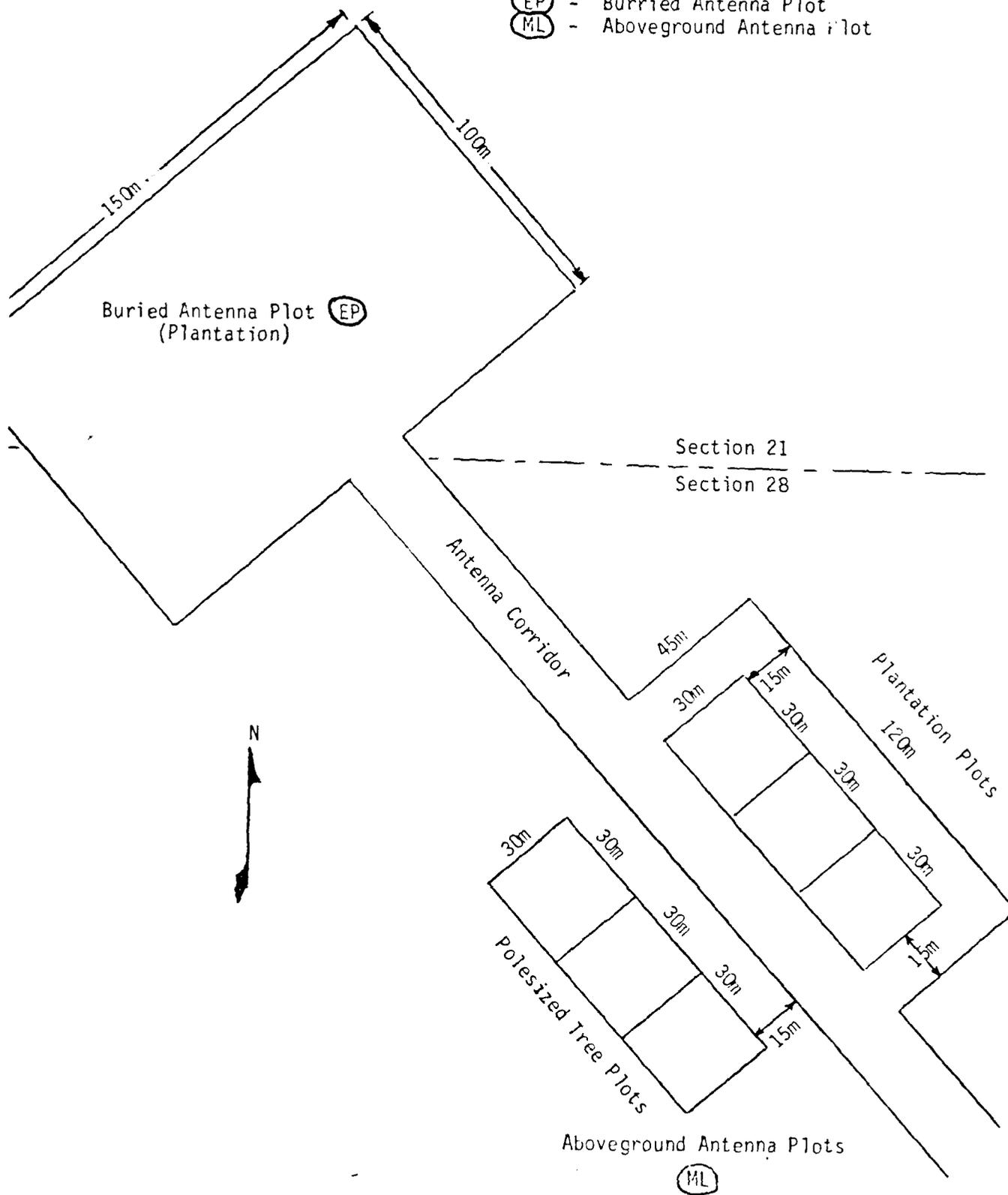
- ⊙ EP - Buried antenna plots
- ⊙ ML - Aboveground antenna plots
- ⊙ GT - Ground terminal antenna plots



ELF ANTENNA PLOTS

T45N, R29W

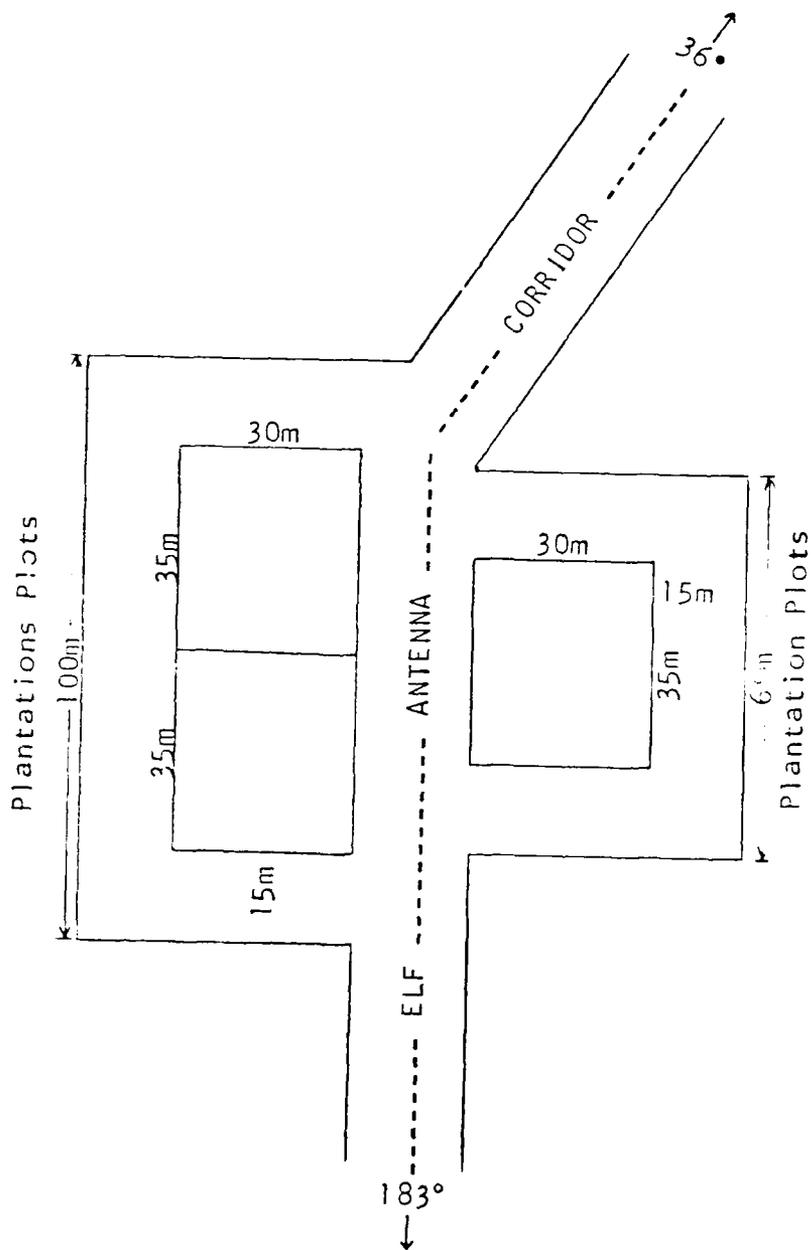
- EP - Burried Antenna Plot
- ML - Aboveground Antenna Plot



GROUND TERMINAL PLANTATION PLOT

Sec. 28, T45N, R29W

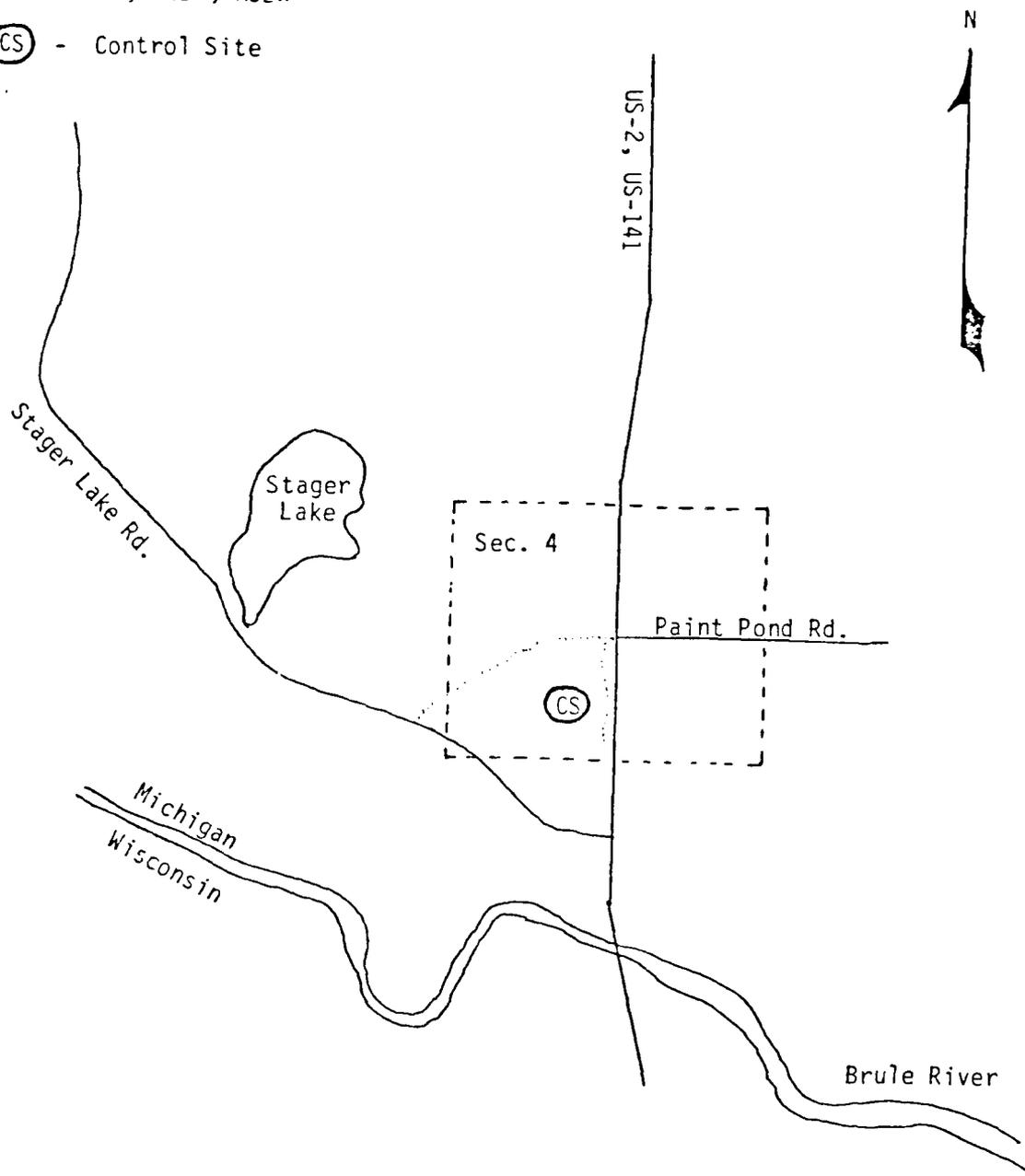
GT



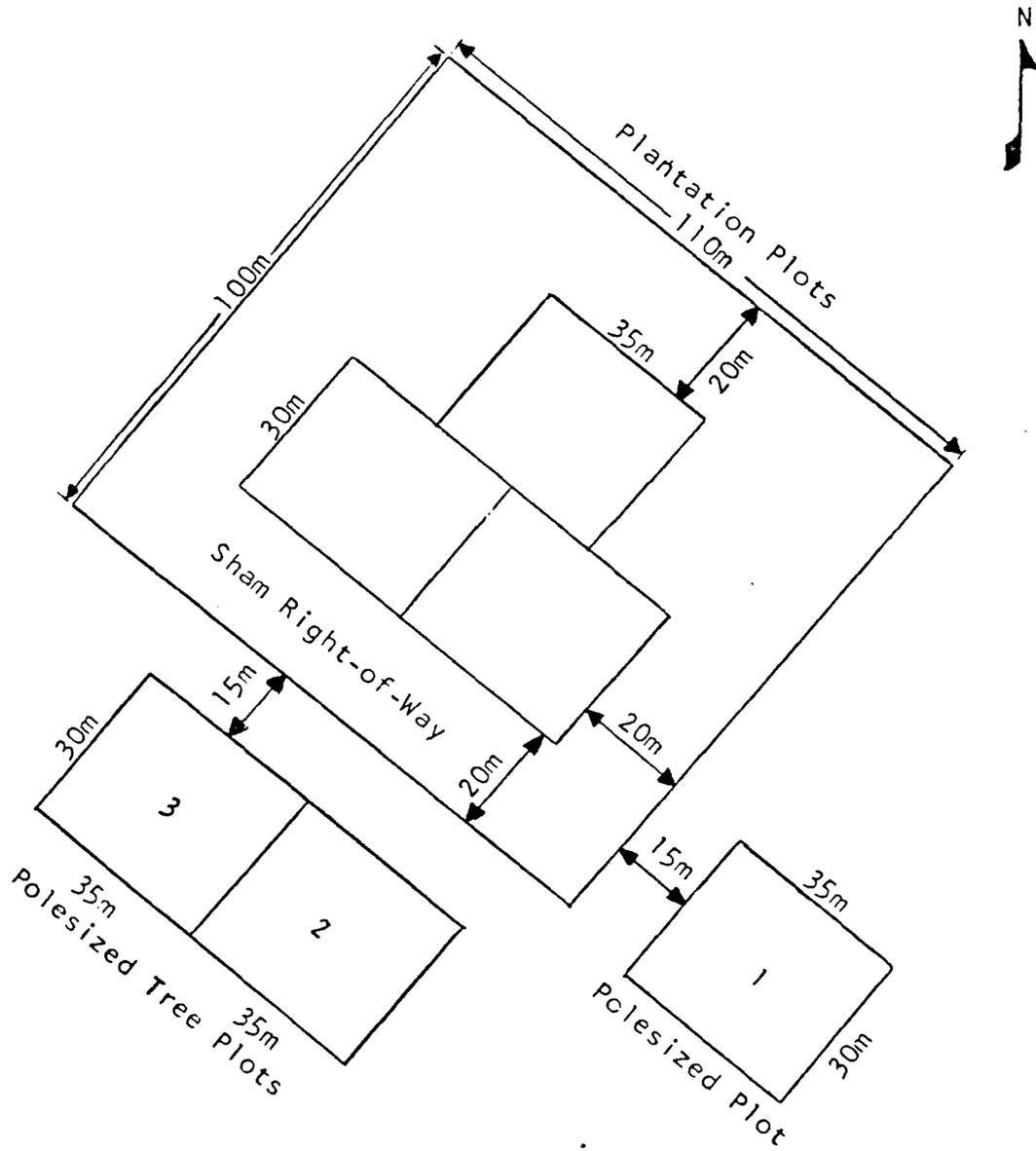
CONTROL PLOT
(Stager Lake)

Sec. 4, T41N, R32W

⊙ - Control Site



CONTROL PLOT - (CS)
(Stager Lake)
Sec. 4, T41N, R32W



APPENDIX B

Summary of the Acer-Quercus-Vaccinum Habitat Type

(Taken from: Coffman, M. S., E. Alyanak, J. Kotar, and J. E. Ferris. 1983. Field Guide, Habitat Classification System for the Upper Peninsula of Michigan and Northeastern Wisconsin. CROFS; Dept. of For., Michigan Technological Univ., Houghton, MI)

ACER-QUERCUS-VACCINIUM HABITAT TYPE (AQVac)

IDENTIFICATION

From key: Trailing arbutus < twice as much coverage as the sum of beaked hazelnut, wild sarsaparilla and barren strawberry. And low sweet blueberry > the sum of wild sarsaparilla, wood betony, twisted stalk, yellow beadlily, false solomon's seal, and spinulose shield fern, with wood betony less than 5% coverage.

Additional Habitat Characteristics:

1. Bracken fern coverage is often greater than 50% on this type and, though variable, averages about 2½ feet and is usually taller than on the Quercus-Acer-Epigaea type.
2. Beaked hazelnut is usually common, often occurring in clumps.
3. Wood betony, twisted stalk, yellow beadlily, false solomon's seal, and spinulose shield fern are often absent or extremely rare on this type. If any of these species are common you may be on the Tsuga-Maianthemum-Vaccinium type.
4. Low sweet blueberry is usually well represented but may be hidden under a cover of bracken fern.

5. Although not as abundant as on the Tsuga-Maianthemum-Vaccinium h.t., white pine commonly occurred on this type before the white pine cuts at the turn of the century, and scattered stumps are still evident today.

CLIMAX OVERSTORY

Dominant: Red Maple, Red Oak
Associate: E. Hemlock, white pine
Minor: Balsam fir, white spruce

SUCCESSIONAL OVERSTORY

See successional diagram as well as detail below.

Succession After Original Logging

1. Logged Climax Stands: Seed origin red maple, red oak, balsam fir, and occasional white pine and white spruce.
2. Logged Successional Stands: sprout red maple, red oak and/or balsam fir, white spruce, white pine, with occasional red pine, jack pine, aspen, or white birch (or clumps of these species).
3. Logged and Burned: aspen/white birch, red pine, jack pine, depending on seed source. Balsam fir and white spruce may be mixed with any of the above species.

UNDERSTORY SPECIES

Table of common and important species in order of decreasing constancy with expected coverage range (**primary indicator, *additional indicator, +common associate):

Constancy	Species	Average Coverage When Present			
		<5%	5-15	15-25	>25%
>75%	Low sweet blueberry		**		
	Bracken fern				+
	Canada blueberry		+		
	Wintergreen		+		
	Large leaved aster		+		
	Beaked hazelnut		**		
	Grasses			+	
75-50%	Pincherry	+			
	Wood anemone	+			
	Juneberry	+			
<50%	Barren strawberry		.		
	Starflower	+			
	Cow wheat	+			
	Wild sarsaparilla	.			
	Sweet fern	+			

SOILS

The AQV habitat type is most common on sandy soils with moderate horizon development. The landform is usually an outwash (lacustrine less common) and may be hilly or dissected. In limited areas this type will occur on heavier soils which are very shallow to bedrock or occur as a shallow cap over sand and gravel.

Appendix C
Soil Profile Description and Laboratory
Data Summary for the Sampled
Pedons on Each Treatment Area

Overhead Antenna

The overhead antenna site is located on a soil of sandy texture, with a large component of medium (.25 to .50 mm) and fine (.075 to .25 mm) sand size fractions. Below a depth of 67 cm the soil contains high amounts of coarse fragments, most of which are stones of greater than 5 cm diameter. Water retention is relatively low, as is characteristic of sandy soils. Chemically, the soil is quite infertile as are most soils in this area. The surface horizon (1-3 cm) contains the greatest amounts of extractable bases, carbon and nitrogen. This layer is also quite acidic, with a pH in water of 4.1. Lower horizons contain minimal amounts of nutrients, and have pH values ranging up to 6.1.

The soil is classified according to Soil Taxonomy as an Entic Haplorthod, sandy, mixed, frigid, due to accumulations of iron and aluminum in the Bs horizons.

Buried Antenna Site

The buried antenna site is on a soil with textures of loamy sand and sand, and has a large component of medium (.25 to .50 mm) and fine (.075 to .25 mm) sand size fractions. High amounts of coarse fragments are found below a depth of 45 cm, with up to 87 percent of the soil below 92 cm composed of coarse materials. Most of these are stones of greater than 5 cm diameter. Water retention is relatively low in this soil. Chemically it is quite infertile, although the finer textured layers show slightly higher levels of extractable bases. The surface horizon (0 to 5 cm) contains the greatest amounts of carbon, nitrogen and extractable bases. The layer is acidic, with a pH in water of 5.3. Lower soil layers have pH values ranging upward to 6.5 in the 2C horizon.

Although the soil has the appearance of an Entic Haplorthod, laboratory

analysis showed that accumulations of iron and aluminum in the Bx horizons were insufficient to meet taxonomic requirements for this classification. Thus, the soil is classified as a Typic Dystrochrept, sandy, mixed, frigid, on the basis of its' ochric epipedon and acid solum. This difference in classification from the overhead antenna site does not indicate significant chemical or physical differences in these soils which would affect productivity.

Control Site

Two soil pedons were sampled at the control site to determine if differences existed between a location at the upper level of the slope and midway down the slope. The pedon at the upper location has textures of loamy sand, loamy fine sand and fine sand to a depth of 66 cm. Below this depth the soil is coarser, with textures of coarse sand and sand. Stones comprise 37 percent of the soil layers between depths of 66 and 102 cm. Water retention capacity is relatively low. Chemically, the soil is quite infertile; however, levels of extractable bases are somewhat higher than expected in soil layers between depths of 36 and 102 cm. The surface horizon contains the greatest amounts of carbon, nitrogen and extractable bases due to enrichment through plant decomposition. This layer is acidic, with a pH in water of 4.6. Lower horizons have pH values ranging up to 6.4.

Although this soil has the appearance of an Entic Haplorthod, laboratory analysis showed that accumulations of iron and aluminum in the Bs horizons were insufficient to meet taxonomic requirements for this classification. Accordingly, the soil was classified as a Typic Dystrochrept, sandy, mixed, frigid, on the basis of its' ochric epipedon and acid solum.

The soil sampled at the midslope position had fewer identifiable soil layers than did the soil at the upper level. Textures were those of loamy

fine sand to a depth of 44 cm, with fine sand below that depth. No coarse materials were found at this location. The water retention capacity of the soil is low, especially at lower depths. Chemically, it is quite infertile. Levels of nitrogen and extractable bases are comparable between the two locations sampled at the control site; carbon values are somewhat lower in the surface layer of this pedon. The surface layer contains the greatest amounts of carbon, nitrogen and extractable bases. It is acidic, with a pH in water of 4.5. Lower horizons have lesser amounts of nutrients, and pH values range upward to 6.5.

The soil at the midslope position was classified according to Soil Taxonomy as an Entic Haplorthod, sandy, mixed, frigid, due to accumulations of iron and aluminum in the Bs horizons.

The two pedons sampled at this site differed mainly in degree of horizon development, with the upper position showing more distinct soil layers. Also, the upper pedon contained stones in the lower horizons while that at midslope did not. Textural differences were apparent in lower horizons, with the upper pedon having a large component of coarse sand. Chemically the two pedons were quite similar.

Composite Samples

Composite samples were taken from each of three study plots on the overhead antenna site and the control site. Core samples were taken, making up three composite samples from each study plot. Data show that most variability occurred between surface horizons and with carbon and nitrogen values. Results of analysis of variance between Bs1 horizons of soils sampled on the two sites are presented in Table 1.

Table 1. Analysis of variance of composite samples of B₁ horizons from the aboveground and control sites.

Analysis	Overhead antenna site Mean	Control site Mean	F-ratio	Significance at 5 percent level
Carbon	.724%	.941%	4.41	N.S.
Nitrogen	.0261%	.511%	5.31	S.
Calcium	.702 meq/100 g	1.059 meq/100 g	1.05	N.S.
Magnesium	.245 meq/100 g	.358 meq/100 g	2.09	N.S.
Sodium	.0084 meq/100 g	.0141 meq/100 g	1.10	N.S.
Potassium	.0647 meq/100 g	.0777 meq/100 g	1.40	N.S.
Acidity	1.011 meq/100 g	1.511 meq/100 g	1.42	N.S.
CEC	6.51 meq/100 g	6.24 meq/100 g	.04	N.S.
pH by CaCl ₂	4.69	4.64	.10	N.S.

Variance in nitrogen content was the only statistically significant difference in chemical values between these two sites. Even though the control site sampled here will not be used in the study, these data show that similar sites can be identified and that studies on these soils will be valid.

Pedon Classification: Entic Haplorthod, sandy, mixed, frigid.
 Soil: overhead antenna site
 Soil No.: R&M1103-1
 Location: Marquette County, Michigan
 Vegetation and Land Use: Northern hardwoods. Forested.
 Parent Material: Outwash over water-worked till.
 Physiographic Position: Rolling upland.
 Topography: Undulating. Gradient is 7 percent. South aspect. Concave. Slope length is
 200 ft.
 Drainage: Well drained.
 Groundwater: Below 160 cm.
 Sampled by: C. Trettin, C. Becker, E. Padley, K. Warren.

(All colors are for moist condition unless otherwise stated.)

0i 2 to 1 cm (1 to .2 inches). Undecomposed hardwood litter.

0a 1 to 0 cm (.2 to 0 inches). Well decomposed hardwood litter; many fine and common medium roots.

A 388 0 to 2 cm (0 to 1 inch). Black (N2/) loamy sand; weak fine granular structure; very friable; many fine and medium, and few coarse roots; very strongly acid; abrupt smooth boundary. (2 to 3 cm thick)

E 389 2 to 13 cm (1 to 5 inches). Pinkish gray (7.5YR 6/2) sand; weak fine granular structure; very friable; many fine and medium, and common coarse roots; 2 percent coarse fragments; strongly acid; abrupt wavy boundary. (5 to 13 cm thick)

Bs1 390 13 to 27 cm (5 to 11 inches). Dark brown (7.5YR 4/4) loamy sand; weak fine subangular blocky structure; friable; many fine and medium, and common coarse roots; 3 percent coarse fragments; strongly acid; abrupt wavy boundary. (12 to 16 cm thick)

Bs2 391 27 to 43 cm (11 to 17 inches). Yellowish red (5YR 4/6) fine sand; weak fine subangular blocky structure; friable; common fine and medium, and few coarse roots; 3 percent coarse fragments; moderately acid; clear wavy boundary. (12 to 19 cm thick)

Bs3 392 43 to 66 cm (17 to 26 inches). Strong brown (7.5YR 5/6) sand; weak fine granular structure; very friable; few fine and medium roots; 1 percent coarse fragments; moderately acid; clear irregular boundary. (22 to 65 cm thick)

2BC 393 66 to 90 cm (26 to 35 inches). Dark brown (7.5YR 4/4) very stony loamy sand; moderate medium subangular blocky structure; friable; few fine and medium roots; 30 percent coarse fragments in stone line at top of till; moderately acid; gradual wavy boundary. (23 to 28 cm thick)

2C 394 90 to 160 cm (35 to 63 inches). Strong brown (7.5YR 4/6) very stony loamy sand; weak fine granular structure; friable; few fine and medium roots; 30 percent coarse fragments; moderately acid.

Pedon Classification: Typic Dystrachrept, sandy, mixed, frigid.
 Soil: Buried antenna site
 Soil No.: R8M1103-2
 Location: Marquette County, Michigan
 Vegetation and Land Use: Northern hardwoods. Forested.
 Parent Material: Outwash.
 Physiographic Position: Rolling Upland.
 Topography: Undulating.
 Drainage: Well drained.
 Groundwater: Below 175 cm.
 Sampled by: C. Trettin, P. Cattelino.

(All colors are for moist condition unless otherwise stated.)

0a 3 to 8 cm (1 to 3 inches). Well decomposed hardwood litter.

A 407 8 to 5 cm (3 to 2 inches). Dark reddish brown (5YR 2.5/2) loamy sand; weak fine granular structure; friable; many fine and medium, and few coarse roots; 3 percent coarse fragments; abrupt wavy boundary. (2 to 6 cm thick)

E 408 5 to 14 cm (2 to 6 inches). Pinkish gray (5YR 6/2) sand; weak fine granular structure; very friable; many fine and medium, and common coarse roots; 3 percent coarse fragments; abrupt wavy boundary. (6 to 23 cm thick)

Bs1 409 14 to 45 cm (6 to 18 inches). Yellowish red (5YR 5/6) sand; weak fine subangular blocky structure; friable; common fine and medium roots; 2 percent coarse fragments; clear wavy boundary. (19 to 38 cm thick)

Bs2 410 45 to 72 cm (18 to 28 inches). Yellowish red (5YR 5/8) sand; weak fine subangular blocky structure; very friable; common fine and few medium roots; 15 percent coarse fragments with a stone line comprised of rounded cobbles; clear wavy boundary. (18 to 24 cm thick)

2Bt 411 72 to 92 cm (28 to 36 inches). Strong brown (7.5YR 4/6) fine sandy loam with few thin reddish brown (5YR 4/4) clay films; medium subangular blocky structure; friable; few fine and medium roots; 50 percent coarse fragments; clear wavy boundary. (9 to 21 cm thick)

2C 412 92 to 175 cm (36 to 69 inches). Dark reddish brown (5YR 3/4) sandy loam; weak fine granular structure; friable; few fine and medium roots; 70 percent coarse fragments.

Buried Antenna Site

SAMPLED AS:

R 83M1-103 -002

FOR AMBIENT MONITORING

GENERAL METHODS 101A, 2A1, 2b

SAMPLE NOS. 05P 407 - 412

DATE 12/25/83
MICHIGAN TECHNOLOGICAL UNIV.
SOIL SURVEY CENTER
SOIL SURVEY LABORATORY
LANSING, MICHIGAN

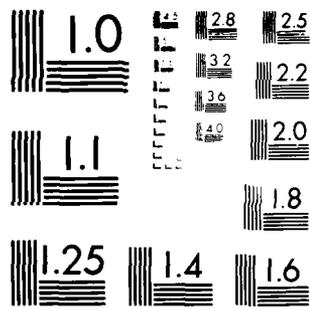
SAMPLE NO.	DEPTH (CM)	GRAIN SIZE (%)	CLAY (%)	SILT (%)	SAND (%)	WATER CONTENT (%)	FIELD MOISTURE (%)	SHRINKAGE (%)	PLASTICITY INDEX	PLASTICITY LIMIT	LIQUID LIMIT	PLASTICITY INDEX	PLASTICITY LIMIT	PLASTICITY INDEX	PLASTICITY LIMIT
83 407	0-5	8.5	5.4	4.3	37.5	3.5	13.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 408	5-15	8.5	5.4	4.3	37.5	3.5	13.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 409	15-30	8.5	5.4	4.3	37.5	3.5	13.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 410	30-45	8.5	5.4	4.3	37.5	3.5	13.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 411	45-60	8.5	5.4	4.3	37.5	3.5	13.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 412	60-75	8.5	5.4	4.3	37.5	3.5	13.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

SAMPLE NO.	UNSATURATED WATER RATIO	FIELD CAPACITY (%)	SHRINKAGE (%)	PLASTICITY INDEX	PLASTICITY LIMIT	LIQUID LIMIT	PLASTICITY INDEX	PLASTICITY LIMIT	PLASTICITY INDEX	PLASTICITY LIMIT
83 407	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 408	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 409	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 410	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 411	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 412	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

SAMPLE NO.	ANNUAL EXTRACTABLE BASES (MEQ/100g)	ALUMINUM (MEQ/100g)	IRON (MEQ/100g)	SODIUM (MEQ/100g)	POTASSIUM (MEQ/100g)	CALCIUM (MEQ/100g)	MAGNESIUM (MEQ/100g)	ZINC (MEQ/100g)	COPPER (MEQ/100g)	MANGANESE (MEQ/100g)	PHOSPHORUS (MEQ/100g)	SULFUR (MEQ/100g)	CHLORINE (MEQ/100g)	BROMINE (MEQ/100g)	IODINE (MEQ/100g)
83 407	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 408	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 409	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 410	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 411	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 412	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

SAMPLE NO.	PERCENTAGE OF ORGANIC MATTER	PERCENTAGE OF HUMUS	PERCENTAGE OF CELLULOSE	PERCENTAGE OF LIGNIN	PERCENTAGE OF STARCH	PERCENTAGE OF PECTIN	PERCENTAGE OF CHITIN	PERCENTAGE OF CHITINASE	PERCENTAGE OF AMYLASE	PERCENTAGE OF CELLULOSE	PERCENTAGE OF LIGNIN	PERCENTAGE OF STARCH	PERCENTAGE OF PECTIN	PERCENTAGE OF CHITIN	PERCENTAGE OF CHITINASE	PERCENTAGE OF AMYLASE
83 407	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 408	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 409	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 410	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 411	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
83 412	1.7	12.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

FAMILY CONTINUOUS SECTION: DEPTH 0-100 PCT CLAY 2.4 PCT 0.1-75MM 84.7. SPECIFIC HUMUS: INDEX OF ACCUMULATED



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

Pedon Classification: Typic Dystrochrept, sandy, mixed, frigid.

Soil: Control Site 3-2-1

Soil No.: R834171-5

Location: Iron County, Michigan

Vegetation and Land Use: Northern hardwoods. Forested.

Parent Material: Outwash.

Physiographic Position: Rolling. Upland.

Topography: Undulating. Gradient is 7 percent. West aspect. Concex. Slope length is 350 ft.

Drainage: Well drained.

Groundwater: Below 180 cm.

Sampled by: C. Trettin, C. Becker, E. Padley, K. Warren.

(All colors are for moist condition unless otherwise stated.)

0i 2 to 0 cm (1 to 0 inches). Undecomposed hardwood litter.

A 395 0 to 4 cm (0 to 2 inches). Black (10YR 2/1) sand; weak fine granular structure; very friable; many fine and medium, and few coarse roots; very strongly acid; abrupt smooth boundary. (3 to 8 cm thick)

Bs1 396 4 to 19 cm (2 to 7 inches). Dark brown (7.5YR 4/4) fine sand; weak fine subangular blocky structure; friable; many fine and medium, and few coarse roots; strongly acid; clear wavy boundary. (5 to 16 cm thick)

Bs2 397 10 to 36 cm (7 to 14 inches). Strong brown (7.5YR 4/6) fine sand; weak medium subangular blocky structure; friable; many fine, common medium, and few coarse roots; moderately acid; clear smooth boundary. (14 to 17 cm thick)

Bs3 398 36 to 66 cm (14 to 26 inches). Strong brown (7.5YR 4/6) sand; massive; friable; common fine, and few medium and coarse roots; moderately acid; abrupt wavy boundary. (30 to 55 cm thick)

Bt 399 66 to 80 cm (26 to 31.5 inches). Reddish brown (5YR 4/4) gravelly loamy sand; weak medium subangular blocky structure; friable; common fine and few medium roots; 15 percent coarse fragments in pebble line at top of horizon; moderately acid; clear irregular boundary. (0 to 15 cm thick)

BC 400 80 to 102 cm (31.5 to 40 inches). Red (2.5YR 4/6) gravelly sand; single grain; loose; few fine roots; 15 percent coarse fragments; moderately acid; abrupt wavy boundary. (7 to 22 cm thick)

C 401 103 to 180 cm (40 to 71 inches). Light brown (7.5YR 6/4) sand; massive; loose; few fine roots; patches of strong brown (7.5YR 5/6) organic staining in top 30 cm of horizon; 1 percent coarse fragments; slightly acid.

Pedon Classification: Entic Haplorthod, sandy, mixed, frigid.
 Soil: Control Site 3-2-2
 Soil No.: R83M171-6
 Location: Iron County, Michigan
 Vegetation and Land Use: Northern hardwoods. Forested.
 Parent Material: Outwash.
 Physiographic Position: Rolling. Upland.
 Topography: Undulating. Gradient is 7 percent. West aspect. Concave. Slope length is 350 ft.
 Drainage: Somewhat excessively drained.
 Groundwater: Below 200 cm.
 Sampled by: C. Trettin, C. Becker, E. Padley, K. Warren.

(All colors are for moist condition unless otherwise stated.)

0i 2 to 0 cm (1 to 0 inches). Undecomposed hardwood litter.

A 402 0 to 6 cm (0 to 2 inches). Black (10YR 2/1) fine sand with few pockets of brown (7.5YR 4/2) E material; weak fine granular structure; very friable; many fine and medium roots; very strongly acid; abrupt smooth boundary. (5 to 10 cm thick)

Bs1 403 6 to 23 cm (2 to 9 inches). Dark brown (7.5YR 4/4) fine sand; weak fine subangular blocky structure; friable; many fine and medium and few coarse roots; strongly acid; clear smooth boundary. (10 to 19 cm thick)

Bs2 404 23 to 44 cm (9 to 17 inches). Strong brown (7.5YR 4/6) fine sand; weak medium subangular blocky structure; friable; common fine and medium, and few coarse roots; slightly acid; clear smooth boundary. (16 to 21 cm thick)

BC 405 44 to 90 cm (17 to 35 inches). Yellowish red (5YR 5/8) sand; massive; loose; few fine roots; slightly acid; gradual smooth boundary. (44 to 48 cm thick)

C 406 90 to 200 cm (35 to 79 inches). Strong brown (7.5YR 5/6) sand with strong brown (7.5YR 4/6) bands at 160 cm; massive; loose; neutral.

Pedon Classification: Entic Haplorthod, Sandy, Mixed, Frigid
Soil: Overhead Antenna Endpoint - Martells Lake
Location: Marquette County, Michigan
Vegetation and Land Use: Northern Hardwoods. Forested.
Parent Material: Outwash
Physiographic Position: Rolling. Upland.
Topography: Undulating. Gradient is 13 percent. Northwest aspect. Convex slope.
Drainage: Well drained.

(All colors are Munsell notation and are for moist conditions)

<u>O_j</u> 2 to 0 cm	Undecomposed hardwood litter.
<u>A</u> 0 to 5 cm	Very dark brown (7.5 YR 3/0) sandy loam. 5 cm thick.
<u>E</u> 5 to 10 cm	Brown (7.5 YR 5/2) loamy sand. 10 cm thick.
<u>B_s</u> 10 to 35 cm	Red (2.5 YR 4/6) loamy sand. 25 cm thick.
<u>B_c</u> 35 to 90 cm	Yellowish red (5 YR 5/6) sand. 55 cm thick.
<u>C</u> 90 to 150 cm	Yellowish red (5 YR 6/6) sand. 60 cm thick.

APPENDIX D

Chemical analyses of composite soil samples obtained from subplots
on the overhead antenna and control sites

APPENDIX E
Laboratory Procedures for Soil Analyses

METHODS OF SOIL ANALYSIS

Analyses of soils were conducted by the Soil Research Laboratory of Ford Forestry Center, Michigan Technological University. Laboratory procedures used were in accordance with those described in the Soil Survey Investigations Report #1 of the Soil Conservation Service, U.S.D.A. (1972). Physical characteristics analyzed included particle size, moisture retention, and natural fabric properties. Chemical analyses performed were for pH, extractable acidity, cation-exchange capacity and extractable bases, and for amounts of organic carbon present. Tests for spodic criteria were also performed where necessary. Descriptions of laboratory methods used are as follows:

Particle Size Distribution Analysis - Pipet Method

Organic matter is removed from the sample by wet digestion with hydrogen peroxide, and soluble mineral material is removed by vacuum filtration. After being dried and weighed, the sample is dispersed in sodium hexametaphosphate solution. The silt and clay are separated from the sand fraction by wet sieving, and placed in suspension in a sedimentation cylinder. The size of the silt and clay fractions are determined by drying and weighing samples of known volume pipetted from the suspension at depths and times which vary with temperature. The sand is dried and divided into five size fractions by dry sieving.

Moisture Retention Analysis (Disturbed samples)

Soil samples are placed in retaining rings on a porous ceramic plate (for 0.1, 1.0, and 3.0 bar extractions) or on a cellulose membrane over a metal screen drain plate (for 15 bar extracting). The plate is covered with water to wet the samples from below, and allowed to stand until the samples are saturated. It is then placed in a pressure vessel, and the samples are desorbed at the required pressure until equilibrium moisture content is

reached (water outflow ceases). The desorbed samples are weighed, oven dried at 105°C, cooled in a dessicator, and weighed again. Moisture content is reported as a percentage of oven dry weight.

Natural Fabric Analyses

Bulk density, linear extensibility, and 1/3 bar moisture retention of the natural soil are measured for those horizons where the thickness and texture allow removal of a natural clod.

The clods are coated with plastic (saran) for water proofing and to prevent disruption. A flat face of the clod is exposed and placed in contact with the surface of a tension table, and the clod is allowed to absorb water until equilibrium is reached at 5 cm of water tension. The saturated clod is then desorbed on a porous ceramic plate in a pressure vessel until equilibrium is reached at .33 bars of pressure.

The exposed face of the clod is then re-coated with saran, and weight and volume are measured. After these measurements are made at 1/3 bar moisture content, the clod is oven dried at 105°C, and the measurements are repeated.

Linear extensibility, moisture content, and also both 1/3 bar and oven dry bulk density are calculated from these measurements, after corrections are applied for the weight of saran. Where analysis of the corresponding disturbed sample has indicated a significant percentage of coarse fragments in the soil, the clod is disrupted and wet sieved to remove the material larger than 2 mm in size. This material is dried and wet sieved to obtain an additional correction factor, since the results of this analysis are meant to apply only to the smaller than 2 mm fraction.

pH Determinations

The water pH of a soil is read in a 1:1 mixture by weight of water and soil. The buffer pH is read in a solution of .01 M Ca Cl₂, using Metrohm-

Brinkman Model 632 pH-Meter.

Extractable Acidity Analysis

A 5 g soil sample is leached with a solution of 0.5 N BaCl₂ and 0.2 N triethanolamine at pH 8.2, followed by a second leaching with a solution of 0.5 N BaCl₂. The resulting solution is titrated to an end point of pH 4.60 with a Metrohm Herisau Titrator E526. Calculations give values of extractable acidity in meq/100 g of soil, on a less than 2 mm basis.

Cation Exchange Capacity and Extractable Bases Analyses

Cations are extracted from a 5 g soil sample with a solution of 1 N NH₄OAc at pH 7.00. The amounts of Ca, Mg, Na and K in solution are determined by atomic absorption spectroscopy using an Instrumentation Laboratories Model 251 AA/AE Spectrophotometer. The soil sample is washed with ethanol, made alkaline with 1 N NaOH, and distilled into 4% boric acid, using the Tecator Kjeltac System 1002 Distilling Unit. The solution is titrated with 0.1 N HCl to an end point of pH 4.60. Calculations from this value show direct cation-exchange capacities in meq/100 g. The cation-exchange capacity is also calculated by the summation method, in which amounts of exchangeable Ca, Mg, Na and K are added to amounts of exchangeable hydrogen, which is determined by the extractable acidity procedure described above.

Carbon (Walkley-Black method)

Organically complexed carbon in a soil sample is oxidized by 1 N K₂Cr₂O₇ with heat generated by addition of concentrated H₂SO₄. The solution is diluted and titrated to an end point of 6.30 mV with 1 N FeSO₄. Calculations show the percent of organic carbon present in soil. This value may be multiplied by 1.72 to obtain percent organic matter in soil.

Spodic Horizon Analyses

Two extractions for Fe and Al are performed to determine whether spodic criteria are met in a soil horizon. The first, a dithionite-citrate extraction, involves shaking a 4 g soil sample overnight in a solution of 0.88% sodium dithionite and 10.94% sodium citrate. In the second extraction, 2 g of soil are shaken overnight in a 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ solution. Amounts of Fe and Al are determined by atomic absorption spectroscopy on the solution extracts, and are reported as percentages.

APPENDIX F

Ambient Monitoring System Configuration
-Data Collection Platforms
-Sensor Types and Location

Ambient System Configuration

Treatment: Control site; Platform #1
 Contains 1 Main Plot, 5 Subplots

<u>Main Plot</u>	<u>Subplots (#1,3-5)</u>	<u>Subplot #2</u>
1 Data Acquisition Unit	2 Soil Temp.	5 Soil Temp.
1 Rain Gauge	2 Soil Mois.	5 Soil Mois.
1 Snow Pillow	1 Air Temp.	1 Air Temp.
1 Air Temp.		1 Rel. Humidity
2 Soil Mois.		
2 Soil Temp.		

Treatment: Overhead Antenna Site; Platform #2
 Contains 1 Main Plot, 5 subplots

<u>Main Plot</u>	<u>Subplots (#1-5)</u>
1 Data Acquisition Unit	2 Soil Temp.
1 Rain Gauge	2 Soil Mois.
1 Snow Pillow	1 Air Temp.
1 Air Temp.	
1 Rel. Hum.	
2 Soil Mois.	
2 Soil Temp.	

Treatment: Burned Antenna Site; Platform #3
 Contains 1 Main Plot, 2 subplots

<u>Main Plot</u>	<u>Subplots (#1-2)</u>
1 Data Acquisition System	2 Soil Temp.
1 Rain Gauge	2 Soil Mois.
1 Snow Pillow	1 Air Temp.
1 Air Temp.	
1 Rel. Hum.	
5 Soil Temp.	
5 Soil Mois.	
1 Radiometer	

APPENDIX G

Equations used in determining whole tree biomass

Equations used in determining whole tree biomass.

<u>Species</u>	<u>Equation (Author)</u>
Bigtooth Aspen	$\text{Ln}Y = .34[.6800+2.2234\text{Ln}(\text{dbh})+.3390\text{Ln}(\text{ht})]^1$ (Young <u>et al.</u> 1964)
Hard Maple	$\text{Log}Y = 3.186+2.35 \text{Log}(\text{dbh})^2$ (Ribe, 1979)
Jack Pine	$\text{Log}Y = 1.0368+2.4206 \text{Log}(\text{dbh})^3$ (Hegy, 1972)
Northern Red Pine	$\text{Ln}Y = 2.57[\text{Ln}(\text{dbh})+0.82]^1$ (Burks, 1981)
Paper Birch	$\text{Ln}Y = .42[.8025+2.2234\text{Ln}(\text{dbh})+.3390\text{Ln}(\text{ht})]^1$ (Young <u>et al.</u> 1964)
Quaking Aspen	$\text{Log}Y = -1.1115+2.3466 \text{Log}(\text{dbh})^3$ (Peterson <u>et al.</u> 1970)
Red Maple	$\text{Log}Y = 3.033+2.466 \text{Log}(\text{dbh})^2$ (Ribe, 1979)
Red Pine	$\text{Ln}Y = 0.8345622+2.4185 \text{Ln}(\text{dbh})^1$ (Winsauer and Steinhilb, 1980)
White Pine	$\text{Ln}Y = .36[.6592+2.2234\text{Ln}(\text{dbh})+.3390\text{Ln}(\text{ht})]^1$ (Young <u>et al.</u> 1964)

¹Y is ODW in pounds, dbh is diameter breast height in inches and ht is height in feet.

²Y is ODW in grams, dbh is diameter breast height in inches.

³Y is ODW in kilograms, dbh is diameter breast height in centimeters.

APPENDIX H

Initial structure of SIR DBMS and description of records
associated with the trees and herbaceous plants task

STRUCTURE OF SIR DBMS
TREES AND HERBACEOUS PLANTS TASK

PLOT

AMBIENT MONITORING DATA	DENDROMETER BAND	TREE MEASUREMENT DATA	100% TREE INVENTORY
HERBACEOUS PHENOLOGY	TREE PHENOLOGY	HERBACEOUS BIOMASS PERCENT COVER	HERBACEOUS BIOMASS EQUATION DATA
MYCORRHIZAL FUNGI COLLECTION	MYCORRHIZA CLASSIFICATION FOUND ON ROOT GROWTH	LITTER TRAP DATA	FOLIAGE SAMPLE DATA

Description of records associated with the trees and herbaceous plants task.
Each recorded in keyed to plot number.

Ambient monitoring data:

Element: 2

Contents: Daily precipitation, air temperature, relative humidity,
soil temperature and soil moisture values.

Collected on: All plots

Collection Cycle: Daily

Dendrometer Band Data:

Element: 3

Contents: DBH measurements on all trees 10 cm and up.

Collected on: Control and aboveground antenna pole-sized plots.

Collection Cycle: Monthly during the growing season.

Tree Measurement Data:

Element: 3

Contents: Yearly tree height, plus additional yearly observations
made on all trees 10 cm and up.

Collected on: Control and aboveground pole-sized plots.

Collection Cycle: Yearly

100% Tree Inventory:

Element: 3

Contents: Tree measurement on all alive and dead trees 2.5 cm and up.

Collected on: Control and aboveground pole-sized plots.

Collection Cycle: Once.

Herbaceous Phenology:

Element: 4

Contents: Dates of various phenological events.

Collected on: Pole-sized aboveground and control plots.

Collection Cycle: Yearly.

Tree Phenology:

Element: 4

Contents: Dates of various phenological events for hardwood and red pine trees.

Collected on: Control and aboveground pole-sized plots for hardwoods. On ground terminal, aboveground and control plantations, plus control and aboveground pole-sized plots for red pine.

Collection Cycle: Yearly.

Herbaceous Biomass:

Element: 5

Contents: Herbaceous biomass measurements.

Collected on: Control and aboveground pole-sized plots.

Collection Cycle: Yearly.

Herbaceous Percent Cover:

Element: 5

Contents: Herbaceous plant coverage measurements.

Collected on: Control and aboveground pole-sized plots.

Collection Cycle: Yearly.

Herbaceous Biomass Equation Data:

Element: 5

Contents: Herbaceous biomass measurements for use in developing herbaceous biomass equations.

Collected on: Control and aboveground pole-sized plots.

Collection Cycle: Once.

Mycorrhizal Fungi Collection:

Element: 6

Contents: Counts of fruiting bodies by isolated mycorrhizal fungi species/categories.

Collected on: All plots.

Collection Cycle: 10-18 times/year.

Mycorrhizal Classification Found on Root Growth:

Element: 7

Contents: Population counts by isolated mycorrhizal fungi species/categories.

Collected on: All plots.

Collection Cycle: Twice/year - early spring and late fall.

Litter Trap Data:

Element: 8

Contents: Nutrient and weight values of litter components.

Collected on: Control and overhead antenna pole-sized plots.

Collection Cycle: Variable - monthly to weekly during growing season.

Foliage Sample Data:

Element: 8

Contents: Nutrient values of leaves.

Collected on: Control and overhead antenna pole-sized plots.

Collection Cycle: Variable - monthly with weekly collection during leaf abscission.

APPENDIX I

Site, tree and soil data from possible study sites that were not
selected for plot establishment.

Site and tree formation collected on 0.27 hectare plots. Values are averages from plots and represent dominant and codominant trees.

Site Information	Sec. 31-A E 1/2 Sec. 31 T45N R28W
Percent Slope	0-13
Aspect Range	None - N-SW
Slope Position	Level, mid-bottom
Elevation	434m

Species	TREE INFORMATION			Sec. 31-A			
	Trees/Ha	Relative Density (%)	Basal Area (m ² /ha)	Site Index	DBH* (cm)	Total Height (m)	Average Age
Bigtooth Aspen	64	10	3.4	67	27.4	22.4	54
Red Maple	211	34	3.4	62	15.0	18.2	44
Paper Birch	155	25	5.2	58	27.7	20.3	60
Jack Pine	38	6	2.3	63	30.9	21.6	54
White Spruce	21	4	1.1	58	28.1	20.9	54
Northern Red Oak	72	11	4.0	64	27.2	21.1	53
Red Pine	38	6	5.7	60	44.9	24.6	68
Eastern White Pine	28	4	1.7	47	36.3	19.6	61
Total	627		27.0				

*Diameter at breast height

Location: Sec. 31-A (E 1/2, Sec. 31, T45N R28W)
Vegetation and Land Use: Northern hardwoods, Forested.
Parent material: Outwash over water-worked till.
Physiographic position: Upland.
Topography: Bottom of slope. Gradient is 8 percent. Aspect is W.
Drainage: Well drained.

(All colors are Munsell notation and are for moist conditions)

<u>O_i</u> 7 to 5 cm	Undecomposed hardwood litter. 2 cm thick.
<u>O_a</u> 5 to 0 cm	Well decomposed hardwood litter. 5 cm thick.
<u>E</u> 0 to 10 cm	Brown (7.5 YR 5/2) loamy sand. 5 percent gravel. 10 cm thick.
<u>B_s</u> 10 to 20 cm	Brown to dark brown (7.5 YR 4/4) sandy loam. 5 percent gravel. 10 cm thick.
<u>B_c</u> 20 to 63 cm	Strong brown (7.5 YR 4/6) sand. 5 percent gravel. 43 cm thick.
<u>C</u> 63 to 150 cm	Brown to dark brown (7.5 YR 4/4) gravelly sand. 87 cm thick.

Site and tree information collected on 0.27 hectare plots. Values are averages from 3 plots and represent dominant and codominant trees.

Site Information		Sec. 31
		S 1/2 Sec. 31 T45N R28W
Percent Slope		0-8
Aspect Range		None - NE
Slope Position		level to mid-slope
Elevation		424 m

Species	Tree Information			Sec. 31			
	Trees/Ha	Relative Density (%)	Basal Area (m ² /ha)	Site Index	DBH* (cm)	Total Height (m)	Average Age
Bigtooth Aspen	316	51	10.0	57	22.3	18.5	51
Red Pine	31	5	2.3	48	40.3	18.3	60
Red Maple	176	29	2.3	51	13.0	14.3	41
Paper Birch	91	15	2.3	50	18.7	15.1	45
Total	614		16.9				

*Diameter at breast height

Location: Sec. 31 (S 1/2 Sec. 31, T45N R28W)
 Vegetation and Land Use; Northern hardwoods. Forested.
 Parent material: Outwash
 Physiographic position: Upland
 Topography: Convex slope. Gradient is 10%. Aspect is NE.
 Drainage: Well drained.

(All colors are Munsell notation and are for moist conditions)

<u>O_j</u> 7 to 5 cm	Undecomposed hardwood litter. 2 cm thick.
<u>O_a</u> 5 to 0 cm	Well decomposed hardwood litter. 5 cm thick.
<u>E</u> 0 to 16 cm	Brown (7.5 YR 5/2) sand. 16 cm thick.
<u>B_s</u> 16 to 38 cm	Yellowish-red (5 YR 4/6) sand. 22 cm thick.
<u>C</u> 38 to 103 cm	Strong brown (7.5 YR 5/6) sand. 65 cm thick.
<u>2C</u> 103 to 133 cm	Strong brown (7.5 YR 5/6) fine sand. 30 cm thick.
<u>3C</u> 133 to 150 cm	Strong brown (7.5 YR 5/6) sand. 17 cm thick.

Site and tree information collected on 0.27 hectare plots. Values are averages from 3 plots and represent dominant and codominant trees.

<u>Site Information</u>		Stagger Creek S 1/2 Sec. 3 T41N R32W
Percent Slope		20-26
Aspect Range		SW-NW
Slope Position		Convex Slope
Elevation		433 m

Species	<u>Tree Information</u>			<u>Sec. 31</u>			
	Trees/Ha	Relative Density (%)	Basal Area (m ² /ha)	Site Index	DBH* (cm)	Total Height (m)	Average Age
Northern Red Oak	131.6	12	5.0	72	22.9	23.0	50
Paper Birch	510.3	46	9.2	60	16.4	19.8	52
Bigtooth Aspen	393.7	35	10.0	65	20.2	21.4	53
Red Maple	83.4	7	1.7	58	16.0	17.0	43
Total	1119.0		25.9				

*Diameter at breast height

Site and tree information collected on 0.27 hectare plots. Values are averages from plots and represent dominant and codominant trees.

Site Information	Sunson Lake NW 1/4 Sec. 1 T45N R28W
Percent Slope	0-11
Aspect Range	W-NW
Slope Position	Crest to mid-slope
Elevation	463 m

Species	Tree Information			Sunson Lake			
	Trees/Ha	Relative Density (%)	Basal Area (m ² /ha)	Site Index	DBH* (cm)	Total Height (m)	Average Age
Bigtooth Aspen	272	31	10.9	73	23.2	22.7	48
Red Maple	242	27	5.2	63	17.1	18.3	43
Paper Birch	312	35	8.0	63	21.0	21.3	56
Northern Red Oak	32	4	1.7	72	27.9	23.0	50
Red Pine	15	2	1.2	63	31.0	21.8	53
Eastern White Pine	10	1	0.6	56	26.7	22.9	56
Total	883		27.6				

*Diameter at breast height

Location: Sunson Lake (NW 1/2 Sec. 1 T45N R29W)
Vegetation and land use: Northern hardwoods. Forested.
Parent material: Water-worked fill.
Physiographic position: Upland. Rolling.
Topography: Slope. Gradient is 10 percent. Aspect is NW.
Drainage: Well drained.

(all colors are Munsell notation and are for moist conditions)

<u>O_i</u> 6 to 4 cm	Undecomposed hardwood litter. 2 cm thick.
<u>O_a</u> 4 to 0 cm	Well decomposed hardwood litter. 4 cm thick.
<u>E</u> 0 to 12 cm	Dark grayish brown (10 YR 4/2) loamy sand. 20 percent gravel. 12 cm thick.
<u>B_s</u> 12 to 32 cm	Dark brown (7.5 YR 4/4) sand. 20 percent gravel. 20 cm thick.
<u>B_c</u> 32 to 44 cm	Brown to dark brown (7.5 YR 4/4) sand. 20 percent gravel. 12 cm thick.
<u>C</u> 44 to 150 cm	Brown to dark brown (7.5 YR 4/4) gravelly sand. 25 percent gravel with occasional stones. 106 cm thick.

Site and tree information collected on 0.27 hectare plots. Values are averages from 3 plots and represent dominant and codominant.

Site Information	Camp 5-A NW 1/4 Sec. 12, T42N R31W
Percent Slope	0
Aspect Range	None
Slope Position	Level
Elevation	-

Species	Tree Information			Camp 5-A			
	Trees/Ha	Relative Density (%)	Basal Area (m ² /ha)	Site Index	DBH* (cm)	Total Height (m)	Average Age
Paper Birch	254	34	6.9	63	20.0	19.2	46
Northern Red Oak	36	5	2.3	66	28.8	21.1	49
Quaking Aspen	396	53	11.5	60	20.1	18.6	47
Balsam Fir	62	8	0.8	36	12.5	11.0	38
Total	748		21.5				

*Diameter at breast height

Site and tree information collected on 0.27 hectare plots. Values are averages from 3 plots and represent dominant and codominant trees.

Site Information	Floodwood - 1 T44N R30W Sec. 15
Percent Slope	3-10
Aspect Range	E-NW
Slope Position	Mid-slope
Elevation	

Species	Tree Information			Floodwood - 1			
	Trees/Ha	Relative Density (%)	Basal Area (m ² /ha)	Site Index	DBH* (cm)	Total Height (m)	Average Age
Bigtooth Aspen	64	14	3.8	65	30.6	22.4	58
Eastern White Pine	13	4	1.5	50	38.8	21.4	66
Red Maple	132	29	3.1	54	18.0	18.6	56
Quaking Aspen	19	4	0.8	58	22.7	19.8	56
Paper Birch	222	49	11.5	64	28.8	22.1	58
Total	450		20.7				

*Diameter at breast height

APPENDIX J

Abstract of paper presented in the Third International Symposium on
Microbial Ecology August 7-12, 1983.

Official Abstract Form

(Read all instructions before typing)

Actinomycetes Associated with Red Pine Mycorrhizae in the Field Versus Nursery Stock. J.N. BRUHN* and S.T. BAGLEY. Michigan Technological University, Houghton, Michigan, U.S.A.

Mycorrhizae aid the mineral nutrition of plants; certain associated actinomycetes have recently also been implicated in this function. Evidence suggests that nursery practices select for less effective mycorrhizal fungus populations than those at many outplanting sites. Therefore, mycorrhizal fungi and associated actinomycetes occurring on red pine in a bare-root nursery and at typical outplanting sites were compared in order to determine the effect of nursery practices on these microorganisms. Rhizosphere soil samples and washed mycorrhizae were analyzed for actinomycete populations; surface-sterilized mycorrhizae were analyzed for mycorrhizal fungus components. Both microbial populations were less diverse under nursery than under field conditions. Species compositions differed between all sites and numbers of species isolated were greater under field conditions. These differences between red pine nursery and field populations of mycorrhizal fungi, in particular, and associated actinomycetes may, in large part, affect seedling survival and productivity at outplanting sites. Management practices should, perhaps, be revised in order to permit selection for, or inoculation of, effective microbiota obtained from the field.

Instructions

Check your poster or slide session preference, complete the check list on the reverse side of this sheet, and sign your name in the space provided.

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LITTER DECOMPOSITION AND MICROFLORA
The Michigan Study Site

ANNUAL REPORT, 1983

SUBCONTRACT NUMBER: E06516-82-C-40015

MICHIGAN TECHNOLOGICAL UNIVERSITY
HOUGHTON, MICHIGAN

ELF COMMUNICATIONS SYSTEM ENVIRONMENTAL MONITORING PROGRAM:
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ANNUAL REPORT, 1983

SUBCONTRACT NUMBER: E06516-82-C-40015

PROJECT MANAGER:


Johann N. Bruhn
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SUMMARY

1

The first full year of experience at the ELF study area has resulted in careful scrutiny of experimental parameters in light of study objectives, refinement of research methods, establishment of considerable baseline data, and appropriate adjustments in work plans for the future.

Work elements 4 and 5, nitrogen cycling and non-mycorrhizal rhizoplane fungi, will be discontinued as the least sensitive indices of environmental perturbation, due to experimental difficulties and budget limitations. Elements 1, 2 and 7, concerning plot selection, ambient monitoring and statistical programming, will also be discontinued for reporting purposes because progress is fully and regularly reported by the complementary herbaceous plant cover and tree studies project. Overhead antenna pole-stand and red pine plantation plots have been located. The antenna ground red pine plantation site has been located and permanent plots will be established there early in the 1984 field season. All planting will take place in 1984. A new control site remains to be selected for approval by IITRI. Ambient monitoring will commence early in the 1984 field season. This project shares the overall experimental design for statistical analysis developed in conjunction with the herbaceous plant cover and tree studies project. The experimental design for this project has been adjusted to characterize parameters at the study plot level rather than the individual tree level.

For 1983-84, studies comprising this project will concentrate on red pine as the sole study tree species for reasons covered in this report. Concentration on red pine does not compromise the sensitivity of these studies to environmental perturbations such as might be caused by ELF fields. Litter decomposition/nutrient flux experiments will utilize both

the pole-stands and plantations and will be closely tied to the herbaceous 2
plant cover and tree studies work on litter production. Rhizoplane
actinomycete population studies will be closely tied to the mycorrhizae
studies in the herbaceous plant cover and tree species project.

A pilot litter decomposition/nutrient flux experiment near the overhead
antenna study sites has permitted evaluation of alternative methodologies
and parameter variability. The paper birch study was discontinued for 1983-
84. Paper birch appears to be poorly suited for these studies because of
large error components of variance associated with its fragile nature. Red
pine decomposition and associated nutrient flux will be characterized by
annual experiments involving monthly retrieval and destructive sampling of
litter samples disbursed in the autumn. Overall mass loss will be
characterized by an individual tethered fascicle method. Nutrient mass flux
will be characterized from bulk litter samples. Both the bulk and tethered
fascicle samples are enclosed in 3mm mesh nylon envelopes, resulting in
comparable data sets. The first operational field experiment is currently
underway at the overhead antenna pole-stand and plantation sites and at the
antenna ground site.

In response to reviewer comments on the first draft of this report, at
least one hardwood species will be included in the study commencing with the
autumn of 1984. Monthly retrieval of materials and inclusion of hardwood
species will take up the budget slack created by discontinuing work elements
on nitrogen cycling per se and non-mycorrhizal rhizoplane fungi.

Trenched plots for study of ammonification and nitrification were
established on the pole-stand study plots at the overhead antenna and
control sites. A single sampling was conducted in October, 1983. No
significant differences in ammonification, nitrification or N₂-fixation were

revealed between the two sites by ANOVA. Statistical analysis indicates that variability in ammonification is low enough to provide moderate precision. Nitrification and N_2 -fixation were more variable. The variability in nitrification may be tied to its sensitivity to low temperatures. The variability in N_2 -fixation has been partially linked to a mechanical processing problem which has been solved. A subsequent sampling in November of 1983 should help determine the precision attainable for this parameter.

Subsequent to the first draft of this report, we have been informed that the plastic barriers employed in trench plot construction would interfere with soil-borne ELF fields. For this reason, as well as due to the relatively high estimates of experimental variation and the need to focus resources on study variables which can be quantified with greater precision, nitrification and ammonification studies have been dropped from this project. For the latter two reasons, nitrogen fixation studies have also been discontinued.

Initial characterization of actinomycetes and heterotrophic bacteria both at ELF study sites and at the Toumey Nursery in Watersmeet has been accomplished. No major differences in densities of heterotrophic bacteria or streptomycetes were detected between ELF study sites or between summer and fall samples. Nursery seedlings and soil were studied in light of the decision to plant red pine on the study sites. Sampling and analytical techniques have been largely worked out. Initial isolation from mycorrhizae and soil will routinely include screening on calcium oxalate agar as well as starch casein agar in light of the possible link between oxalate utilization by actinomycetes and the nutritional role of mycorrhizae. No streptomycetes were isolated from surface-sterilized mycorrhizal root tips. Therefore, future work will concentrate on washed roots and rhizosphere soil.

Comparisons between nursery and field populations revealed no major differences in overall numbers or numbers of types of heterotrophic bacteria or actinomycetes in soil samples. However, washed roots from the nursery yielded larger overall numbers of fewer types of heterotrophic bacteria and streptomycetes than did field-collected roots. This suggests that selective conditions occur in the nursery ecosystem and shows that population trends or disturbances can be detected using these methods. Five of the nine streptomycete types isolated in the nursery were isolated only in the nursery. The predominant nursery type produces red pigment in starch casein agar and was not found in field-collected samples. This streptomycete strain will serve a key role in studying population shifts when nursery seedlings are planted in the field. Concentration on red pine should not compromise sensitivity to environmental disturbance. Field isolations from red pine generally yielded more types of streptomycetes than did paper birch, and many of these types were found at all three study sites sampled. In order to provide as sensitive and meaningful an index to environmental disturbance as possible, sampling in 1984 will characterize actinomycete associations with specific mycorrhiza morphology types.

The litter decomposition subsystem of any forest ecosystem serves to 1) transform the essential plant nutrients in organic matter into forms available for root uptake, 2) pool the nutrients collected by primary producers, and 3) release these nutrients in a regulated fashion for re-use by the autotrophs. The energy provided by litter decomposition also fuels heterotrophic dinitrogen fixation and capture of nutrients washed from the atmosphere or leached from living plants. Due to the large quantities of potentially available plant nutrients found in the litter component of forest biomass, knowledge of key decomposition processes and their rates is essential to conceptualization of ecosystem dynamics. Organic matter decomposition is primarily accomplished by heterotrophic microorganisms whose activities are regulated by the environment. Recognizing the delicate balance of ecosystem functioning, it is apparent that environmental factors which disrupt decomposition processes detract from the optimum flow of nutrients to vegetation. As one such environmental factor, ELF electromagnetic fields merit investigation for possible effects on the litter decomposition subsystem.

Litter decomposition is a complex process involving a variety of organisms engaged in the degradation of a wide range of organic compounds. The primary agents of organic matter decomposition are the fungi and bacteria. Within these broad groups, a relatively small cadre are responsible for degradation of complex structural materials such as cellulose and lignin. Among the fungi, cellulose and lignin degradation are accomplished by members of the Basidiomycetes and Ascomycetes, including their imperfect states. Of the bacteria, members of the Actinomycetes have been found to degrade cellulose (Crawford 1978, Teslinova et. al. 1981)

and lignin/lignocellulose (Sutherland et. al. 1979, Antai and Crawford 1981) in both coniferous and deciduous litter systems (Goodfellow and Dawson 1978, Kauri 1978, Goodfellow and Cross 1974, Andrews and Kenerly 1979, Antai and Crawford 1981, Ogawa et. al. 1981).

Rates of litter breakdown by the soil microbiota are strongly affected by the status and cycling of nitrogen in the organic materials (Kelly and Henderson 1978, VanLear 1980). In natural ecosystems, atmospheric N₂ is converted into forms useful to plants through dinitrogen fixation. In order to become available for plant uptake, resulting organic nitrogen complexes must first undergo transformation to ammonium (ammonification) by the soil microflora. In many ecosystems, such as the aspen-mixed conifer cover type found in the proposed ELF system study area, ammonium is rapidly converted to nitrate (nitrification) by a relatively select group of soil bacteria (Vitousek and Melilla 1979).

The broad objectives of this study are: 1) to characterize a) the rates of foliar organic matter decomposition and nutrient cycling and b) populations of mycorrhiza-associated actinomycetes on selected sites within the ELF antenna area prior to operation of the antenna, and 2) to use these baseline data to evaluate possible ELF field effects on these sensitive processes and populations. Although the two remaining work elements represented in this project (reflected in 1a and 1b above) may appear disjointed, the relevance of each element is readily apparent when viewed in conjunction with the Herbaceous Plant Cover and Tree Species project as an integrated whole.

Over the period since the 1982 Annual Report was prepared, several developments have necessitated reevaluation and restructuring of the original study plan. The most far-reaching change, affecting both the litter decomposition/nutrient flux and actinomycete work elements, involves

the shift in emphasis from paper birch and red pine as study species to red 7
pine alone for 1983-84. This shift was necessitated by 1) a like shift in
the emphasis of complementary mycorrhiza and root growth studies in the
herbaceous plant cover and tree studies project, and 2) low levels of
precision attainable in characterizing paper birch litter
decomposition/nutrient flux. Actinomycete studies will be shifted from
mature pines on or near study sites to planted seedlings in 1984 for
coordinated sampling with the mycorrhiza characterization and root growth
study (element 7) in the herbaceous plant cover and tree studies project.
Litter decomposition/nutrient flux work for 1984-85 will 1) include at least
one hardwood species, 2) involve monthly retrievals of litter materials, 3)
be conducted on both pole-stands and plantations, and 4) characterize
decompositon/nutrient flux at the site rather than the plot level. The
1983-84 experiment currently in the field will involve monthly sampling at
the site level, rather than more intensive sampling on a biannual basis at
the plot level as originally planned.

Another major change is the deletion of work elements 4 and 5,
"nitrogen cycling" and "non-mycorrhizal fungi." As a result of efforts
during 1982-83 and the current budget restrictions, these elements will be
discontinued as the least sensitive of the chosen parameters in detecting an
environmental influence of ELF electromagnetic fields. Continuation of
these elements could compromise the remaining elements by draining needed
resources.

Rejection of the second proposed control site by IITRI late in the 1983
field season due to unacceptable differences in levels of 60 Hz fields
between the proposed control and treatment sites, has commensurately set
back progress in characterizing a control site. Hence, the 1983-84
decomposition/nutrient flux experiment characterizes only the overhead
antenna and ground antenna study sites.

Element 1: Plot Selection

Progress in study plot selection is fully documented in the annual report prepared for the Herbaceous Plant Cover and Tree Studies project, subcontract number E06516-82-C-10015. This work element will not be continued in the Litter Decomposition and Microflora studies project as of November 1, 1983. It should be understood, however, that continued input to the plot selection process from this project will ensure selection and establishment of study plots appropriate to both projects. Priority items will be selection of a control study site and establishment of control and endpoint study plots.

Element 2: Development, Installation and Operation of the Ambient Monitoring System

Progress on this work element is also fully related in the annual report prepared for the Herbaceous Plant Cover and Tree Studies project, subcontract number E06516-82-C-10015. This work element will also be discontinued from the Litter Decomposition and Microflora studies project as of November 1, 1983. Input from this project will continue, nevertheless, to ensure collection of appropriate ambient monitoring data to serve the needs of this study. Of special importance will be daily summaries of air temperature, precipitation, and surface soil temperature and moisture content.

Element 3: Litter Decomposition/Nutrient Flux

Introduction

Overall litter mass loss has traditionally been used as a measure of fully integrated litter decomposition (Kendrick 1959, Jensen 1974, Millar 1974). It has been shown, however, that both the accuracy and precision of mass loss as a sensitive index of organic matter deterioration declines with time beyond approximately one year, while nutrient flux provides continuously meaningful ecological information (Stark 1972, Graustein et. al. 1977, Bruhn et. al. 1979, Knutson et. al. 1980, Bruhn 1981). Microfloral population shifts have been shown to influence the rate of overall litter decomposition (Mitchell and Millar 1978). Conversely, overall litter mass loss and nutrient flux are useful measures of the impact of environmental perturbations on the integrated activities of the litter biota. ELF fields represent one possible cause of perturbations.

Litter decomposition/nutrient flux studies greatly extend the usefulness of litter productivity data collected in the course of forest vegetation studies. Knowledge of litter biomass production and nutrient content likewise serves as the basis for decomposition study. Further, the study methods employed integrate the activities of microflora with those of all but the largest arthropods and earthworms, extending the value of all population data.

Since the 1982 Annual Report was written, an entire year's experience with red pine and paper birch litter decomposition and nutrient flux has been gained through the pilot study at Martels Lake. Experience gained has resulted in significant restructuring of this work element.

The most significant change involves the species of litter to be studied. Efforts for 1983-84 are concentrated solely on red pine for two reasons. First, it has been decided that mycorrhiza studies will be

concentrated solely on pine. Second, birch foliage proved to be relatively ¹⁰
poorly suited to quantitative study of weight loss and nutrient flux due to
its fragile nature. Further details are provided under Methods and
Results. We believe that concentration on red pine does not reduce the
sensitivity of this work element to possible effects of ELF fields on the
litter decomposition subsystem. Nevertheless, in response to reviewer
comments on the first draft of this report, at least one hardwood species
will be included in future experiments, commencing in the autumn of 1984.
The intensified sampling required will be made possible by discontinuing
work elements on nitrogen cycling and non-mycorrhizal rhizoplane fungi.

It has also been decided to shift statistical emphasis from
characterization of litter fall on a biannual basis beneath individual trees
to characterization of litter fall on a monthly basis at the study site
level.

Litter decomposition is being quantified as percent change over time in overall mass and nutrient (N, P, K, Ca, Mg, S) masses. Analysis of litter nutrient content is being conducted by the Soils Analysis Laboratory, Department of Forestry, Michigan Technological University. Fresh-fallen red pine and paper birch foliar litter was collected in the autumn of 1982 near Martels Lake for incorporation into a pilot decomposition/nutrient flux experiment designed for estimation of parameter variability. In addition, the pilot study tested the relative merits of estimating overall mass loss of red pine foliar litter via bulk litter envelopes vs. individually tethered perfect fascicles in envelopes. Fascicles offer the advantages that 1) errors due to litter losses from and inputs to envelopes over the course of an experiment do not occur, and 2) fascicles broken during the course of an experiment can be discarded prior to analysis. Sixty bulk litter envelopes (3mm nylon mesh, 22cm x 28cm) each containing 10 g of fresh-fallen red pine or paper birch foliar litter and 12 envelopes (3mm nylon mesh, 22cm x 14cm) containing 10 pre-weighed perfect fresh-fallen red pine needle fascicles each were placed in the field near Martels Lake in December, 1982. Half of these envelopes were retrieved after snowmelt in May of 1983, the remainder in October. Sample sizes necessary to detect spatial/temporal differences at desired probability levels were calculated from estimated variances.

Responding to the results of the Martels Lake pilot study, the first full-scale decomposition/nutrient flux experiment was established in early December, 1983, at the overhead antenna and antenna ground sites. No control site was included because our tentative control site was disqualified by IITRI late in the field season and an alternate site remains to be established. The 1983-84 decomposition experiment is restricted to red pine due to the relatively low levels of precision attainable in

characterizing paper birch litter decomposition/nutrient flux. Red pine litter for the 1983-84 experiment was collected from the LaCroix red pine plantation near Houghton due to 1) its proximity to MTU, and 2) its relative remoteness from interfering electromagnetic fields. A single parent collection at one location avoids differences which might be present in substrate quality between different stands on different sites.

Beginning with the 1983-84 experiment, red pine weight loss will be studied by the tethered fascicle method, while nutrient flux will be studied with bulk litter samples. Fresh:dry mass ratios and initial nutrient content were determined for random samples taken from the LaCroix plantation parent collection. Replicate mesh envelopes containing random subsamples from the parent litter collection were placed for the winter and for one year on each of nine study plots (three plots each at the overstory pole-stand and plantation sites and at the endpoint plantation site). Four bulk litter envelopes (each containing 10 g of the parent collection) and two envelopes containing 10 perfect preweighed needle fascicles each were disbursed at five random locations on each of the nine study plots (three plots each at the overhead antenna pole-stand and plantation and at the antenna ground plantation). Sample sizes were based on results of the 1982-83 pilot study at Martels Lake, and will be adjusted if necessary for the 1984-85 experiment based on results of the experiment currently underway.

Responding to reviewer comments on the first draft of this report, samples currently in the field will be retrieved on a monthly basis during 1984 in a manner which characterizes the three sites rather than the nine plots on those sites. Sufficient samples will be collected each month to permit both 1) analysis of variance and co-variance between dates, sites and litter species, and 2) fitting of single and/or double exponential decay models to estimate constants which describe mass loss over time (Wieder and Lang 1982).

Table 1 presents a summary of the litter decomposition/nutrient flux data for the pilot over-winter experiment at Martels Lake. The tethered fascicle approach to measuring pine foliage decomposition proved much more accurate than the bulk sample method. It has been calculated that approximately 37 fascicles per plot would suffice to detect 1.0 percent over-winter mass loss differences between sample populations ($= .01$). Bulk litter samples lost more mass than individual fascicles, suggesting that more needle fragments were lost than were gained by litter envelopes. Chemical analysis of bulk litter samples was completed for five elements: N, P, K, Ca and Mg. Analysis of S content awaits return of the nitrogen analyzer, which was defective on arrival. Samples have been saved for this purpose. Calculations suggest that 10 pine envelopes per plot would suffice to detect over-winter differences of one order of magnitude between sample populations in percent N, P, K, Ca and Mg by mass ($= .05$).

Fragmentation of birch leaf litter, even over the course of a single winter, resulted in a tendency toward over-estimation of mass loss. On the other hand, extraneous matter which became attached to birch leaves over time could not be effectively removed without damaging the litter and contributed to excessive variability in nutrient flux as well as weight loss determinations. Fifteen bulk samples per study plot would be required to detect a 1.0 percent over-winter mass loss difference between sample populations ($= .05$). Calculations also indicate that 22, 17, 4, 3, and 2 bulk samples would be required to detect over-winter differences of one order or magnitude between sample populations in percent Ca, K, N, Mg and P by mass ($= .05$), respectively.

Table 2 presents a summary of the litter decomposition/nutrient flux data for the pilot one-year experiment at Martels Lake. Mean mass loss for

individually tethered red pine fascicles was 20.4 percent, a substantial increase from 8.5 percent over winter. Calculations show that 36 tethered fascicles per study plot should permit detection of 1.5 percent one year mass loss differences between sample populations ($\alpha = .01$). Taking into account the possibility of needle breakage as well as the fact that conditions may vary between sites, 100 fascicles (50 each over winter and one year) were disbursed at each ELF study plot in December, 1983, for the first operational litter decomposition experiment. Bulk pine envelopes again overestimated mass loss, losing 26.1 percent of their mass. The difference between methods is undoubtedly due to loss of needle fragments from envelopes. For instance, one tethered fascicle was eliminated from analysis because one needle was broken. Though the broken section was probably little more decomposed than the rest of the fascicle, weight loss would have been recorded as 50.2 percent.

Chemical analysis of the 60 one-year bulk pine and birch samples has been completed, but statistical analysis has not yet been performed.

Overall and nutrient mass loss figures derived from the pilot experiment are extremely encouraging for red pine study. Significant decomposition is shown to take place over winter. Decomposition over winter and after one year in the field is definitely well enough advanced to offer a sensitive index for detection of environmental perturbation, especially when analyzed in conjunction with ambient monitoring data collected at the study plots.

Beginning with the 1983 ^{2nd} experiment, nutrient mass loss will be statistically analyzed instead of final nutrient content. Initial nutrient content of litter samples in the field has been determined from the parent litter collection.

Table 1. Data summary for the over-winter segment of the pilot litter decomposition/nutrient flux experiment (1982-83) at Martels Lake.

Sample Type	Sample Size (Number)	Percent Mass Loss (W/W)	Final Percent (W/W)A											
			\bar{X}	S ²	N	\bar{X}	S ²	P	\bar{X}	S ²	K	\bar{X}	S ²	Ca
Bulk Pine Envelopes	30	10.97	2.70	0.46	0.06	0.04	0.002	0.11	0.02	0.55	0.029	0.09	0.005	
Tethered Pine Fascicles	60	8.53	4.98											
Bulk Birch Envelopes	30	11.00	3.30	0.85	0.073	0.10	0.006	0.53	0.11	1.25	0.29	0.26	0.01	

A based on analysis of 22 pine samples and 21 birch samples.

Element 4: Nitrogen Cycling

Introduction

Nitrogen (N) is unique among plant nutrients because of its presence almost entirely in organic forms. No inorganic reserve is normally present to alleviate nitrogen losses to volatilization or leaching. In natural ecosystems, atmospheric N_2 is converted into forms useful to plants through dinitrogen fixation. In order for this N to become available for plant use, organic nitrogen complexes in microbial and plant tissues must be mineralized to ammonium by the soil microflora. In many ecosystems, the ammonium is then biologically converted to nitrate.

The diverse group of soil organisms participating in the various segments of the nitrogen cycle are affected by the same factors which influence overall decomposition of organic matter: kind and nutrient content of organic matter; soil moisture and temperature; and populations of soil micro- and macro- fauna and flora (Witkamp and van der Drift 1961, Bartholomew 1965). These same soil organisms are extremely sensitive to environmental perturbations, such as timber harvesting, air pollution, acid rain, and heavy metal additions (Jurgensen 1979, Matson and Vitousek 1981). Since different segments of the microflora are active in dinitrogen fixation, ammonification, and nitrification, rates of these nitrogen transformation would be excellent indicators of soil microorganisms' responses to ELF fields.

Methods

Nitrogen mineralization occurring during organic matter decomposition was monitored using the trenched plot technique. Five trenched areas (2x2 m) were established on each study plot by cutting off all roots to a depth of 30 cm with a spade. Plastic was inserted into the spade slit to prevent root regrowth into the trenched areas. A composite sample of three 10 cm

diameter cores was taken from the litter layer in each trenched plot.

Ammonium and nitrate determinations were performed on the litter samples within 24 hours after collection. Samples were then dried at 105⁰C for dry weight and moisture content determination.

Nitrogen fixation rates were estimated using the acetylene reduction technique. Four composite samples of the litter layer were taken from five areas selected randomly on each study plot and placed into 1/2 pint mason jars (a total of 20 samples per plot). Three of the jars were injected with acetylene to give an approximate acetylene concentration of 10% (V/V). The remaining replicate was not injected with acetylene to serve as a control to monitor endogenous ethylene production.

The jars were buried in the litter layer and, after a 22 hour incubation period, gas samples were obtained using Vacutainer vials. These samples were analyzed for acetylene and ethylene concentrations on a gas chromatograph. Litter samples were dried at 105⁰C for dry weight and moisture content determinations.

The delay in locating suitable ground antenna and control sites during the summer allowed sampling of ammonium and nitrate concentrations and nitrogen fixation only in October (Tables 3 and 4). While it would have been desirable to have obtained more samples to estimate parameter variability and error estimates, the number obtained was sufficient to gain an estimate of suitable sampling levels.

An analysis of results by ANOVA techniques showed no significant differences in ammonium and nitrate levels or nitrogen fixation rates between sites. The error estimate of ammonium and nitrate at the above ground site was 2.9 ppm and 0.9 ppm, an error percent of 17.1 percent and 34.6 percent, respectively. The error value for ammonium is acceptable considering the heterogeneous composition of the litter layer. An additional ten composite samples per plot (45 samples/site in total) would be required to give ammonium estimates within 10 percent of the mean. The increase in analysis cost and labor effort does not seem to be warranted for such a small reduction in error estimate. The error estimate for ammonium was similar at the control, but this has no bearing since a new control site will be used next year.

The error estimate for nitrate was considerably higher than for ammonium. Nitrification is carried out by a relatively select group of soil bacteria, and thus would be more sensitive to microsite conditions than ammonification. However, the nitrifying bacteria are quite sensitive to low soil temperatures, and the high variability exhibited may be related to sampling in October.

Similar to soil nitrate concentrations, nitrogen fixation rates also showed a considerable error estimate at the above ground antenna site, 47.1 percent. Again, similar to nitrification, nitrogen is fixed by a relatively

Table 3. Ammonium and Nitrate Concentrations in the Litter Layer at the Above-ground Antenna and Control Sites

<u>Site</u>	<u>NH₄</u> (ppm)	<u>NO₃</u> (ppm)	<u>Moisture Content</u> (%)
Aboveground Antenna	17.0	2.5	72.0
Control	9.2	4.1	55.3

Table 4. Nitrogen Fixation, as Estimated by Acetylene Reduction Activity, in the Litter Layer at the Above-ground Antenna and Control Sites

<u>Site</u>	<u>Ethylene Produced</u> (nmoles)	<u>Moisture Content</u> (%)
Aboveground Antenna	2.5	80.3
Control	4.4	103.8

small group of bacteria. However, the high error value is likely due to a problem in processing the samples in the field rather than to large microsite variations. Apparently, one of the needles used to inject acetylene into the sample jars became partially plugged. This would affect acetylene concentrations in the jars and, consequently, the amount of ethylene produced. A later sampling conducted in November will be used to calculate a new error estimate.

It was brought to our attention at the annual E.L.F. Environmental Monitoring Program Subcontractors Meeting in March, 1984, that the barrier material used in constructing trench plots and the orientation of the trench plots themselves will influence soil-borne E.L.F. fields. For this reason, trench plot studies have been discontinued. Because of the relatively great variability encountered and the need to focus available resources on study variables which can be quantified with greater precision, the entire nitrogen cycling work element has been discontinued.

Element 5. Non-mycorrhizal Rhizoplane Fungi

Introduction

Population changes resulting from environmental perturbations have been documented for various fungi. Fungal populations respond to air pollutants including acid rain, and to heavy metal addition. Study of rhizoplane fungi would tie decomposer population studies back to the dominant vegetation. Because of the shallow occurrence of a major proportion of feeder roots, sampling from the organic horizons would tie in closely with the rest of the decomposition-related studies.

Methods

A subsample of the mycorrhizal root tips collected at each sampling period was washed and plated onto water agar for isolation of non-mycorrhizal root-associated fungi. Colonies which developed were hyphal-tipped into pure culture on potato dextrose agar for identification and description of population dynamics.

Results

Approximately 53 non-mycorrhizal fungi have been isolated into pure culture from field and nursery red pine roots. Of these, 32 represent readily identifiable genera. Most of the remainder failed to produce structures in culture which would permit rapid routine recognition. Resources available will not permit the intensity of study which would be required in order to characterize these populations satisfactorily.

To date, species representing the following genera have been identified: Alternaria, Arthrotrichum, Aspergillus, Botryotrichum, Cephalosporium, Chloridium, Cladosporium, Cylindrocarpon, Dicyma, Gliocladium, Gliomastix, Gliotrichum, Mortierella, Penicillium, Rhizopus, Spicaria, Staphylotrichum, Trichoderma, and Verticillium. Many of these genera can be characterized as heavy sporulators and/or as containing species dependent in large part on relatively simple carbohydrate energy sources. Such fungi are likely to be less important in the scheme of decomposition/nutrient flux processes than fungi which degrade more complex substrates such as cellulose and lignin. Identification of isolated fungi to species within these genera would be time-consuming on a routine basis. Finally, population estimates for heavy sporulators are more a function of their reproductive potential than of their actual activity. The ecological roles played by the unidentified isolates is naturally unclear, but projected resources for this project will not permit elucidation of the matter. Because the suitability of these fungal populations to detection of environmental perturbation remains undetermined and sufficient funding is not available to clarify this point, work in this area has been discontinued.

Element 6. Rhizoplane and Rhizosphere Actinomycetes

Introduction

Litter decomposition is a complex process involving a variety of organisms engaged in the degradation of a wide range of organic compounds. The primary agents of organic matter decomposition are the fungi and bacteria. A wide variety of heterotrophic bacteria (HB) may be present in both the rhizosphere (i.e., root-influenced soil) and the rhizoplane (i.e., root surface), carrying out an equally wide variety of degradative and biosynthetic reactions. Of these bacteria, the actinomycetes in general, and the streptomycetes in particular, may specifically be involved in the degradation of complex molecules such as cellulose and lignocellulose (Alexander 1977, Crawford 1978). These activities aid in litter decomposition, cycling of nutrients, and, in the case of calcium oxalate degradation (Knutson et al. 1980), act directly to affect mycorrhizal nutrition.

As a group, actinomycetes are routinely found in soil. The subgroup that has been best studied and characterized is the genus Streptomyces (i.e., the streptomycetes). These bacteria (HB) are similar to the fungi in having mycelial growth and reproducing by asexual spores. Because of this, the presence of streptomycetes may not be as subject to minor variation in soil/root growth conditions as are the unicellular HB. Streptomycetes can also routinely be identified to genus level simply by their appearance on primary isolation media (Kützner 1981). In addition, most studies on "actinomycetes" in the rhizoplane/rhizosphere have dealt primarily with streptomycetes (Andrews and Kenerley 1979, Goodfellow and Dawson 1978, Knutson et al. 1980). For these reasons, particular attention was paid to detection and characterization of streptomycete types in the various

rhizosphere and rhizoplane samples studied. Because of their reproduction by spores (unlike most of the HB), streptomycete densities can not really be quantitatively determined using serial dilution or plate count techniques (Alexander 1977, Kützner 1981). However, the different types present in different samples can be determined, with their relative incidence (i.e., number of colonies per type) providing an indication of each type's activity/dominance.

Sensitivity of tree root/mycorrhizae studies will be enhanced through the use of the rhizoplane as the sampling universe for decomposer populations. Mycorrhizae are known to interact functionally with some of the rhizoplane HB and streptomycetes (Jayasuriya 1955, Knutson et al. 1980). Rhizosphere bacterial populations are also under direct influence of the tree roots, although to a lesser extent than the rhizoplane populations. Knowledge of the rhizoplane/rhizosphere HB and streptomycete populations in pre-ELF samples will increase the likelihood of ultimately detecting any root-associated ELF effects. Study of the rhizoplane, in particular, also ties decomposition studies back to the dominant vegetation. Finally, due to the shallow occurrence of a major proportion of feeder roots, sampling from the organic horizons ties in closely with the other decomposition-related work being conducted as part of this study.

Methods

All rhizosphere soil and root samples were collected and prepared by team researchers in the MTU Forestry Department and delivered to the Environmental Microbiology lab in the Department of Biological Sciences in sterile containers. These samples for bacteriological analysis were processed within 24 hours of receipts (and usually within 4 hours). Soil and root samples were designated (and analyzed) according to tree type, i.e. birch, red pine or balsam fir.

Soil samples were first sieved through a 2 mm mesh screen, in order to remove roots, rocks, etc. One-gram (wet weight) portions of each soil sample were then weighed out and placed into 9.0 ml sterile dilution blanks (containing 0.01 M phosphate buffer, pH 7.2). Subsequent serial dilutions were made using the same type of sterile buffer. A larger portion of the sieved soil was transferred to a pre-weighed aluminum pan and weighed; this portion was then placed in a drying oven for soil dry weight determination.

Washed root samples were handled in a somewhat similar manner, except that additional precautions were taken to maintain aseptic conditions. Using flame-sterilized forceps, 0.1 g (wet weight) of washed roots was placed in 9.9 ml sterile phosphate buffer and homogenized in a flame-sterilized 30 ml blender. This mixture was then transferred to a sterile, screw-cap test tube; further dilutions were made in sterile phosphate buffer. Washed root dry weight determinations were made in the same manner as for the soil samples.

Early in the study (i.e. Fall, 1982), several surface-sterilized root samples were also examined. Sample preparation was the same as for the washed root samples.

All soil and root samples (after preparation and appropriate serial dilution) were spread-plated onto starch casein agar (SCA) in 9.0 cm petri

dishes, a medium designed to enhance isolation of actinomycetes, and streptomycetes in particular (Kützner 1981). Cycloheximide (50 mg/l) and nystatin (50 mg/l) were added to the SCA to prevent fungal growth (Andrews and Kennerly 1979, Goodfellow and Dawson 1978). Three to four dilutions (in duplicate) were spread-plated per sample. All plates were incubated at 20°C. Total numbers of heterotrophic bacterial colonies, plus streptomycete colonies specifically, were determined at approximately weekly intervals until there was negligible increase in colony number (i.e. 14-21 days).

During the first part of this study, a second isolation technique was tested. This technique was reported to increase the isolation of streptomycetes and other actinomycetes (Hirsch and Christensen 1983). The samples were prepared and serially diluted as listed above, but membrane filtration was used instead of spread-plating. The authors reported that actinomycetes, being filamentous, would grow through the filter pores and form colonies on the SCA medium. After a growth period (at 20°C), the filters were removed from the SCA plate surfaces. Actinomycete colonies then developed on the SCA plate surfaces upon re-incubation.

Attempts were also made to isolate calcium oxalate degrading bacteria (including streptomycetes) directly from test soil and roots, using the medium and technique of Knutson et al. (1980). These spread-plates were prepared, incubated, and counted similar to the SCA plates.

After approximately 7 and 14 days incubation, strain diversity estimates were made on colonies growing on SCA. With the exception of streptomycete colonies, all colonies were characterized for form, elevation, margin, surface, opacity, texture, diameter (in mm), and pigmentation (Gerhardt 1981). All colonies with the same characteristics were considered to represent one type or strain. At least one colony per type was isolated

in pure culture for further characterization. Additional tests on these isolates included gram, catalase, and oxidase reactions, cellular morphology, endospore formation, growth on MacConkey agar, growth at 10, 35, and 45°C, growth at pH 4, 7, and 9, growth in the presence and absence of oxygen, motility, oxidative/fermentative metabolism (with glucose), and fermentation/utilization of various carbon and energy sources. Although complete bacterial characterization of the soil and root samples was not the intent of these studies, the above data were useful to 1) verify the strain/type designation, 2) help determine if actinomycetes other than Streptomyces were present in the samples, and 3) provide a potentially useful record of heterotrophic bacterial types for comparison between samples and sampling times (given that a select group of bacteria were isolated using SCA). Some of these non-streptomycete isolates were identified using Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons 1974).

Streptomyces colonies could readily be identified on SCA because of their mycelial growth. The numbers of these bacteria were recorded (and reported) separately. Initially, all streptomycete colonies per sample were transferred from SCA and isolated in pure culture. Later in the study (i.e., Fall, 1983), only one colony for each of the types found in a sample was isolated in pure culture. Using the format of Shirling and Gottlieb (1966), the streptomycete cultures were characterized for melanin production, color of aerial mycelia, production and color of reverse and soluble pigments, sporophore structure, and carbohydrate utilization. Additional tests were conducted to evaluate calcium oxalate (Jayasuriya 1955, Knutson et al. 1980), cellulose, and lignocellulose (Crawford 1978) degradation. Some of the Streptomyces cultures were identified to species using the scheme of Küster (1972). As electron microscopic examination of

sporophore/spores was not possible, the identification were only tentative. However, sufficient data were obtained to separate the Streptomyces cultures into distinct types (having similar characteristics).

Results

Soil and root samples from a wide variety of sites/tree types were analyzed for heterotrophic bacteria (HB) and streptomycete densities and types from November, 1982, to October, 1983 (Table 5). The samples from the Martels Lake area were used to check isolation/enumeration/identification techniques. This site was not sampled in later studies as it was not close enough to the final ELF system location. A range of ELF-related soil and root samples (associated with red pine and paper birch) were analyzed during the summer of 1983, primarily because the control, ground antenna, and overhead sites were moved after analysis of already collected samples had commenced. By the fall sampling period, both sampling sites and analysis techniques were well established. Four sets of red pine soil and root samples from the Toumey Nursery were also analyzed to provide comparisons of in situ bacterial populations and background data for the future red pine seedling outplanting program.

Heterotrophic Bacteria and Streptomycete Densities. - Viable counts for HB in general, and streptomycetes in particular, are presented in Tables 6 through 10 for samples analyzed during 1982-83. The HB and streptomycete densities in the first red pine and birch soil, red pine roots, and one balsam fir soil sample from Martels Lake are presented in Table 6. As mentioned above, these samples were used to establish techniques and expected ranges and types of bacteria. The numbers of HB and streptomycete types provide estimates of population diversity. Although this site was not used in the 1983 studies, some general observations can be made. The first is that, although SCA is somewhat selective for streptomycetes, HB outnumbered streptomycetes by 10 to 100-fold. Secondly, fewer numbers of HB were associated with the washed roots (i.e., rhizoplane) vs. the rhizosphere soil; streptomycete densities were about equal, perhaps because of

Table 5. Numbers of soil and root samples analyzed during 1982-1983.

Sampling Period	Sample Location	Sample Type Tested		
		Soil	Washed Roots	Surface-Sterilized Roots
Fall, 1982 - Winter, 1983	Martels Lake			
	Red Pine	3	4	4
	Paper Birch	1		
	Balsam fir	1		
	Nursery	1	1	1
Summer, 1983	Control (first), Paper Birch	1	3	
	Control (second), Paper Birch	1	3	
	Overhead, Paper Birch	2	3	
	Endpoint, Red pine	2	6	
	Control (first), Red Pine	1	3	
	Control (second), Red Pine	1	3	
	Overhead, Red Pine	2	4	
	Nursery	3	3	
	Control (second), Paper Birch	3	2	
	Overhead, Paper Birch	3	3	
Fall, 1983	Control (second), Red Pine	4	4	
	Endpoint (second), Red Pine	3	3	
	Overhead, Red Pine	3	3	
	Overhead, Red Pine	3	3	

Table 6. Heterotrophic bacteria and streptomycetes associated with Martels Lake red pine, balsam fir, and paper birch roots and rhizosphere soil (Fall - Winter, 1982).

Tree Species	Collection	Viable Counts ^{A,B}					
		Soil		Washed Roots		Sterilized Roots	
		Heterotrophic Bacteria ^C	Streptomycetes	Heterotrophic Bacteria ^C	Streptomycetes	Heterotrophic Bacteria ^C	Streptomycetes
Red Pine	Nov., 1982	4.2×10^7 (12)	2.4×10^5 (5)	8.9×10^6 (6)	3.7×10^4 (2)	$< 3.0 \times 10^5$ (4)	$< 1.0 \times 10^3$ (0)
	Nov., 1982	3.5×10^8 (8)	1.7×10^6 (3)	3.0×10^7 (B)D 1.9×10^7 (W) (7)	1.0×10^4 (B) 4.0×10^6 (W) (2)	1.9×10^5 (B) 2.3×10^4 (W) (6)	$< 1.0 \times 10^3$ (B) $< 1.0 \times 10^3$ (W) (0)
Balsam Fir	Nov., 1982	1.5×10^7 (8)	8.1×10^5 (3)	3.8×10^6 (7)	1.9×10^6 (3)	1.6×10^4 (4)	$< 1.0 \times 10^3$ (0)
Paper Birch	Nov., 1982	3.5×10^6 (7)	2.3×10^5 (3)	NT ^D	NT	NT	NT
	Nov., 1982	$> 6.5 \times 10^7$		NT	NT	NT	NT

A Reported per g dry weight of sample and mean of two replica plates.

B Numbers in parenthesis indicate the number of different stain types identified per sample.

C Includes numbers of streptomycetes.

D B - Black root tips; W - White root tips; NT - Not Tested.

their ability to produce spores. More types of HB and streptomycetes were generally found in soil as compared to washed roots.

Several attempts were made to isolate streptomycetes from surface-sterilized red pine roots from the Martels Lake site. As indicated in Table 6, HB were isolated in lower numbers and with fewer types than with the washed roots; however, no streptomycetes were detected in any of the sterilized root samples at the lowest dilution that could be examined. This indicates that the streptomycetes were associated with the exterior rather than the interior of the roots. Therefore, surface-sterilized roots were not examined in the 1983 studies.

Attempts to selectively isolate streptomycetes/actinomycetes using the filtration technique of Hirsch and Christensen (1983) were generally unsuccessful. Using methods given in a presentation at the 1982 American Society for Microbiology Annual Meetings, virtually no bacteria of any type were detected. Therefore, this technique was not used in any later studies. (Note: the authors altered the procedure in their late-1983 publication, making this method perhaps more feasible for use in future studies).

As indicated earlier, the ELF overhead, control, and endpoint sampling sites were moved during the summer, 1983, sampling period. HB and streptomycete densities associated with washed roots from both the old and new sites are presented in Tables 7 and 8 for red pine and birch samples, respectively. One red pine soil sample was analyzed for each of the three new ELF sites (Table 7) and one birch soil sample for each of two of the new sites (Table 8); no birch samples were collected at the endpoint sites, as this site will be cut over and planted with red pine.

As noted with the 1982 red pine samples, the HB outnumbered the streptomycetes 10 to 100-fold in both the red pine and paper birch samples. Some general differences were observed between tree species. In the red

Table 7. Heterotrophic bacteria and streptomycetes associated with washed red pine roots and rhizosphere soil, (Summer 1983).

Sample Tree	Sample Location	Date Collected	Viable Counts A,B			
			Soil		Washed Roots	
			Heterotrophic Bacteria	Streptomycetes	Heterotrophic Bacteria	Streptomycetes
Pine 1	Endpoint	7/18			1.85x10 ⁶ (10)	1.38x10 ⁵ (5)
Pine 2	Endpoint	7/18			8.29x10 ⁵ (12)	1.23x10 ⁴ (4)
Pine 3	Endpoint	7/18			1.20x10 ⁶ (9)	1.04x10 ⁵ (5)
Composite	Endpoint	7/18	2.82x10 ⁷ (9)	9.20x10 ⁵ (3)		
Pine 4	Overhead	7/18			5.06x10 ⁵ (9)	1.52x10 ⁴ (5)
Pine 5	Overhead	6/22			8.78x10 ⁷ (7)	--- (1)
Pine 6	Overhead	6/22			6.34x10 ⁶ (12)	6.96x10 ⁵ (5)
Composite	Overhead	7/18	2.52x10 ⁶ (9)	9.23x10 ⁴ (4)		
Pine 7	Control (first)	6/23			1.25x10 ⁷ (7)	1.83x10 ⁵ (3)
Pine 8	Control (first)	6/23			2.84x10 ⁶ (6)	3.97x10 ⁴ (2)
Pine 9	Control (first)	6/23			5.74x10 ⁶ (7)	7.50x10 ⁴ (1)
Pine 10	Control (second)	7/19			4.96x10 ⁵ (10)	4.86x10 ⁴ (3)
Pine 11	Control (second)	7/19			1.48x10 ⁶ (5)	1.45x10 ⁵ (2)
Pine 12	Control (second)	7/19			1.21x10 ⁵ (6)	3.57x10 ⁴ (4)
Composite	Control (second)	7/19	6.76x10 ⁶ (8)	1.94x10 ⁵ (3)		

A Reported per g dry weight sample and mean of two replica plates.

B Numbers in parenthesis indicate the number of different strain types identified

C Includes numbers of streptomycetes.

Table 8. Heterotrophic bacteria and streptomycetes associated with birch roots (washed) and rhizosphere soil, Summer 1983.

Sample Designation	Sample Location	Date Collected	Viable Counts A,B			
			Soil		Washed Roots	
			Heterotrophic Bacteria	Streptomycetes	Heterotrophic Bacteria	Streptomycetes
Birch-5	Control (first)	6/23			5.35x10 ⁷ (8)	1.96x10 ⁶ (1)
Birch-6	Control (first)	6/23			2.65x10 ⁷ (9)	3.18x10 ⁶ (3)
Birch-7	Control (first)	6/23			4.14x10 ⁷ (7)	2.14x10 ⁶ (3)
Birch Composite	Control (second)	7/19				
Birch-8	Control (second)	7/20	4.93x10 ⁶ (7)	3.45x10 ⁵ (3)	3.05x10 ⁷ (8)	1.78x10 ⁶ (2)
Birch-9	Control (second)	7/20			5.64x10 ⁷ (9)	2.03x10 ⁶ (4)
Birch-10	Control (second)	7/20			3.00x10 ⁶ (8)	1.64x10 ⁵ (4)
Birch Composite	Overhead	7/18				
Birch-2	Overhead	6/22	5.43x10 ⁶ (7)	1.62x10 ⁵ (3)	4.62x10 ⁷ (12)	4.33x10 ⁶ (2)
Birch-3	Overhead	6/22			7.31x10 ⁷ (7)	1.96x10 ⁵ (1)
Birch-4	Overhead	6/22			8.22x10 ⁷ (5)	4.29x10 ⁵ (-)

A Reported per g dry weight sample and mean of two replica plates.

B Numbers in parenthesis indicate the number of different strain types identified per sample.

C Includes numbers of streptomycetes.

pine samples (Table 7), the HB levels were lower on the washed roots than in the soil; in the same samples, the streptomycete densities were approximately the same. In contrast, there was little difference in density for HB or streptomycetes between the birch soil and washed root samples. Generally, higher densities of HB and streptomycetes were associated with birch washed roots; this was not unexpected, in part because of the difference in composition between deciduous and coniferous roots (Knutson et al., 1980). The red pine and birch soils had approximately the same number of HB and streptomycete types, as did the washed roots. There was no apparent difference in HB and streptomycete densities between the washed roots at the red pine endpoint and control sites or between the birch control and overhead sites. Only one sample was obtained from the red pine overhead site.

The samples analyzed during fall, 1983 (Tables 9 and 10) provide the best data for comparison between rhizosphere and rhizoplane and between sites. The same general trends were observed for HB and streptomycete densities and ratios in soil and on roots for red pine (Table 9) and paper birch (Table 10) as were found with the summer, 1983, samples. There did not appear to be major differences in HB or streptomycete densities between the red pine sites or between the summer and fall collections.

Viable count data from red pine nursery soil and washed root samples are listed in Table 11. One sterilized root sample was analyzed, but no streptomycetes were detected. HB and streptomycete densities in nursery soil were about the same as for the red pine soil from the field (Tables 7 and 9). There were increases in HB and streptomycete, in particular, densities on the washed roots. These increases and the observed decreases in HB and streptomycete types (compared to the field samples), indicate that selective conditions exist in the nursery; this can cause the classical

Table 9. Heterotrophic bacteria and streptomycetes associated with red pine roots (washed) and rhizosphere soil, Fall 1983.

Sample Designation	Sample Location	Date Collected	Viable Counts ^{A, B}			
			Soil		Washed Roots	
			Heterotrophic Bacteria ^C	Streptomycetes	Heterotrophic Bacteria ^C	Streptomycetes
Pine 1	Endpoint	Oct. 1983	7.58x10 ⁶ (3)	1.43x10 ⁶ (3)	1.86x10 ⁶ (13)	2.20x10 ⁵ (7)
" 2	Endpoint	"	1.18x10 ⁷ (14)	3.57x10 ⁶ (7)	4.74x10 ⁶ (10)	4.74x10 ⁵ (5)
" 3	Endpoint	"	1.13x10 ⁷ (11)	1.88x10 ⁶ (6)	3.13x10 ⁶ (10)	4.20x10 ⁵ (3)
" 4	Overhead	"	3.40x10 ⁷ (9)	7.08x10 ⁵ (2)	3.08x10 ⁷ (7)	3.83x10 ⁵ (2)
" 5	Overhead	"	2.10x10 ⁷ (7)	7.00x10 ⁵ (1)	1.57x10 ⁶ (9)	2.48x10 ⁵ (5)
" 6	Overhead	"	2.03x10 ⁷ (12)	8.76x10 ⁵ (8)	8.74x10 ⁵ (12)	1.28x10 ⁵ (6)
" 10	Control	"	4.46x10 ⁶ (5)	8.25x10 ⁴ (1)	6.39x10 ⁵ (16)	4.05x10 ⁴ (6)
" 11	Control	"	1.05x10 ⁷ (10)	2.58x10 ⁵ (4)	2.64x10 ⁶ (12)	1.98x10 ⁵ (5)
" 12	Control	"	3.04x10 ⁶ (8)	3.30x10 ⁵ (5)	7.30x10 ⁵ (6)	9.50x10 ³ (3)
" 13	Control	"	7.00x10 ⁵ (9)	9.17x10 ⁴ (4)	2.97x10 ⁵ (12)	5.09x10 ⁴ (4)

A Reported per g dry weight sample; mean of two replica plates.

B Numbers in parenthesis indicate the number of different strain types identified per sample.

C Includes numbers of streptomycetes.

Table 10. Heterotrophic bacteria and streptomycetes associated with birch roots (washed) and rhizosphere soil, Fall 1983.

Sample Designation	Sample Location	Date Collected	Viable Counts ^{A,B}			
			Soil		Washed Roots	
			Heterotrophic Bacteria ^C	Streptomycetes	Heterotrophic Bacteria ^C	Streptomycetes
Birch 2	Overhead	Oct. 1983	8.28×10^6 (7)	5.32×10^5 (3)	4.88×10^7 (5)	3.68×10^5 (1)
" 3	Overhead	"	1.75×10^7 (12)	2.68×10^6 (6)	3.47×10^7 (18)	4.86×10^5 (9)
" 4	Overhead	"	1.33×10^7 (8)	1.18×10^7 (3)	6.92×10^7 (11)	1.80×10^7 (6)
" 8	Control	"	4.95×10^6 (8)	1.02×10^6 (4)	5.78×10^7 (12)	2.74×10^6 (3)
" 9	Control	"	5.31×10^5 (3)	1.18×10^5 (2)	2.85×10^6 (11)	7.80×10^4 (2)
" 10	Control	"	7.30×10^7 (3)	1.22×10^5 (-)		

A Reported per dry weight. sample; mean of two replica plates.

B Numbers in parenthesis indicate the number of different strain types identified per sample.

C Includes numbers of streptomycetes.

Table 11. Heterotrophic bacteria and streptomycetes associated with nursery red pine roots (washed) and rhizosphere soil.

Sample Designation	Date Collected	Viable Counts ^{A,B}					
		Soil		Washed Roots		Sterilized Roots	
		Heterotrophic Bacteria ^C	Streptomycetes	Heterotrophic Bacteria ^C	Streptomycetes	Heterotrophic Bacteria ^C	Streptomycetes
1	Feb. 1983	5.10×10^7 (8)	4.00×10^5 (3)	2.60×10^7 (7)	4.90×10^6 (2)	1.50×10^6 (5)	$< 1.00 \times 10^3$ D(0)
1	July 1983	2.94×10^6 (9)	6.00×10^5 (3)	1.52×10^7 (2)	1.09×10^7 (2)		
2	"	3.75×10^6 (6)	1.21×10^5 (2)	4.20×10^7 (7)	1.47×10^7 (2)		
3	"	5.10×10^6 (5)	$< 1.20 \times 10^5$ (-)	1.38×10^5 (6)	$< 1.00 \times 10^4$ (-)		

A Reported per g dry weight sample; mean of two replica plates.

B Numbers in parenthesis indicate the number of different strain types identified per sample.

C Includes numbers of streptomycetes.

D No streptomycete colonies at lowest sample volume tested (i.e., 10^{-3})

response of a decrease in types or diversity with an increase in abundance of those types which are present.

These viable count data show the variability that is inherent when dealing with such a complex system as soil or even macerated roots (Alexander 1977). However, these pre-ELF viable counts indicate the expected ranges of HB and streptomycete densities (using serial dilution on SCA) under unperturbed conditions. The differences between ELF study site and nursery red pine samples show that trends or disturbances can be detected. The influence of soil pH on HB and streptomycete incidence has not yet been determined; soil pH values will be available upon completion of the soil characterization study being conducted as part of the Herbaceous Plant Cover and Tree studies project.

Streptomycete and HB Types and Identification. - Detailed descriptions were recorded for HB and streptomycete colony types, along with the incidence of a particular type per sample. Additional biochemical and staining tests with HB and streptomycete pure cultures generally indicated the validity of using morphological criteria as means of estimating diversity (i.e., cultures of one morphotype had similar biochemical characteristics). There were a few instances of overlap with streptomycete types, resulting in apparently fewer types per sample or site than expressed in Tables 6 through 10. Complete records have been made of HB and streptomycete type characteristics to aid in typing of future samples. Pure cultures are also being maintained of all streptomycete and most HB types for future studies and for comparisons with types found in future samples.

This study was not designed to characterize all the HB in the rhizosphere and rhizoplane samples nor to identify of all the HB types. The types of HB detected were somewhat biased by use of SCA, which meant that not all types of HB in the soil or root samples could necessarily be

recovered. However, there were no noticeable differences in types of HB (excluding streptomycetes) isolated from any of the soils or from any of the washed roots. Some differences were detected between types of HB associated with soils vs. those associated with washed roots. This difference may be due in large part to variations in types and quality of available nutrients. The majority of the HB types were gram-negative rods, such as Pseudomonas spp. Several gram-positive rods and cocci, such as Bacillus spp., Micrococcus spp., Staphylococcus spp., and Corynebacterium spp., were also present (in both the soil and washed root samples). One of the predominant organisms from the sterilized root samples was Chromobacterium violaceum, which was not detected in other types of samples. All these HB are considered to be normal soil inhabitants (Alexander 1977).

Data from characterization of streptomycetes are presented in Appendix A. The type designations in this table are based on a standardized typing scheme using media other than SCA (Shirling and Gottlieb 1968) and are not based on the original morphological characteristics on SCA. These types can only tentatively be identified to species using the scheme of Kuster (1972). The actual identifications may change with the use of a forthcoming, simplified scheme (John Sutherland, Personal Communication). These tentative identifications are as follows: type 1 - S. vinocetus; type 2 - S. prunicolor; types 3 and 15 - S. aburaviensis; type 4 - S. fulvoviridis; type 5 - S. pseudogriseolus; type 7 - S. spiroverticillatus; type 9 - S. pilosus; type 13 - S. flavescens; type 16 - S. aureofacicules; type 17 - S. aureomonapodiales; type 19 - S. flocculus; type 20 - S. mosanionsis; type 21 - S. griseoalbus; and type 23 - S. cyanocolor. Types 6, 8, 10, 11, 12, 14, and 22 require further tests and/or spore morphology determinations before even tentative identification can be made. An additional streptomycete, S. collinus, was isolated only from the first red

pine samples from the Martels Lake site.

Roughly 50 percent of the streptomycete types were able to utilize calcium oxalate as a sole source of carbon and energy (Appendix A), but only 4 of these were able to clear the calcium oxalate agar. These tests are being refined and repeated. Appendix B presents a more complete description of the calcium oxalate degradation studies with streptomycetes and HB. Very few of the streptomycete types tested were able to utilize cellulose and/or lignocellulose. All the streptomycete types are being (re)tested with new formulations of these media.

The associations of these streptomycete types with the soil and root samples tested from November, 1982, to October, 1983, are summarized in Table 12. The unique conditions at the nursery are clearly shown as only four of the nine types found with red pine nursery roots and soil were found in any of the field site samples; types 1, 2, 4, 21, and 23 were only found with the nursery samples. There was considerable overlap in incidence of streptomycete types with the field red pine and paper birch samples, i.e., only three types were not found associated with both tree species. The most commonly detected streptomycetes (although not necessarily the most numerous) were types 3, 5, 15, and 22. Fifty percent of the streptomycete types found in the Martels Lake red pine samples were not isolated from any further samples.

Streptomycete type associations specifically with soil and/or washed roots from red pine or birch sites, field or nursery, and summer or fall, 1983, are presented in Tables 13 through 17. For the ELF sites, the tables are divided into control, endpoint, and overhead. Where multiple samples were collected per site, these were combined; more types may have been present at a site than actually found in individual samples. The predominant streptomycete type at each site is also indicated.

Table 12. Presence of streptomycete types in all samples (soil and washed root) collected during 1982-1983.

Streptomycete Type Number ^B	Presence per sampling site ^A										Nursery	Martels Lake
	Red Pine		Overhead		Birch		Overhead		Nursery	Martels Lake		
	Control	Endpoint	Control	Overhead	Control	Overhead	Control	Overhead				
1											X	
2											X	
3	X	X		X		X		X				X
4											X	
5	X	X		X		X		X			X	
6											X	
7				X								
8	X	X		X		X		X				
9												
10						X		X				
11	X	X		X		X		X				
12	X	X		X		X		X				
13												X
14	X	X		X		X		X				X
15	X	X		X		X		X				X
16						X		X				X
17												X
18	X							X		X		X
19												X
20	X			X				X				X
21											X	
22	X			X				X				X
23											X	

^AComposite of all samples.

^BType numbers correspond to colony morphology descriptions in Appendix A.

Table 13. Association of Streptomyces types with red pine site samples, Summer 1983.

Streptomyces Type Number	Presence per Sampling Site ^A							
	Control		Endpoint		Overhead			
	Soil	Roots	Soil	Roots	Soil	Roots	Soil	Roots
3	X ^C	X ^C	X	X ^C				
5		X		X			X	
6							X	
7				X ^C				
9			X ^C					
11	X	X		X			X	
12	X	X	X		X			
14		X						
15	X						X	
16							X	
18		X						
20							X	
22	X	X		X			X	

A Composite of all samples.

B Type numbers correspond to colony morphology descriptions in Appendix A.

C Predominant type.

Table 14. Association of streptomycete types with birch site samples, Summer 1983.

Streptomycete Type Number ^B	Presence per Sampling Site ^A					
	Soil		Control		Overhead	
	Washed	Roots	Washed	Roots	Washed	Roots
3	X ^C	X ^C	X ^C	X ^C	X ^C	X ^C
5	X	X	X	X	X	X ^C
8			X			
9			X			
10			X			
11						X
14			X			X ^C
20			X			
22	X	X	X	X	X	X

A Composite of all samples.

A, B Type numbers correspond to colony morphology descriptions in Appendix

C Predominant type.

Table 15. Association of streptomyces types with red pine site samples, Fall 1983.

Streptomycete Type NumberB	Presence per Sampling SiteA					
	Control Soil Washed Roots	Endpoint Soil Washed Roots	Overhead Soil Washed Roots	Control Soil Washed Roots	Endpoint Soil Washed Roots	Overhead Soil Washed Roots
3	X	X	X ^C	X	X	X
5	X	X ^C	X	X	X	X
8	X					
9		X		X		
11	X		X ^C		X	X
12			X		X	X
15	X	X	X ^C	X	X ^C	X ^C
20	X ^C					
22	X	X ^C	X	X	X	X

A Composite of all samples.

B Type numbers correspond to colony morphology descriptions in Appendix A.

C Predominant type.

Table 16. Association of streptomycete types with birch site samples, Fall 1983.

Streptomycete Type Number B	Presence per Sampling Site A					
	Control		Overhead		Washed Roots	
	Soil	Washed Roots	Soil	Washed Roots	Soil	Washed Roots
3	X	X ^C	X ^C		X	
5		X			X	
8		X ^C	X			
9				X		
11	X	X		X		
12					X	
15		X			X	
18		X			X ^C	
22				X		X

A Composite of all samples.
 B Type numbers correspond to colony morphology descriptions in Appendix A.
 C Predominant type.

Table 17. Association of streptomycete types with nursery red pine samples.

Streptomycete Type Number ^B	Presence per Sampling Site ^A	
	Soil	Washed Roots
1	X	X
2	X ^C	X ^C
4	X	X
5	X	
6		X
18	X	
21	X	
23	X	X

A Composite samples

B Type numbers correspond to colony morphology descriptions in Appendix A.

C Predominant type.

Tables 13 through 16 (red pine and paper birch on ELF study sites, summer and fall) generally show that more types of streptomycetes were associated with red pine than with birch samples, that the predominant types for soil or washed root samples were also found with the corresponding soil or washed root sample (although not always predominant), and that a large proportion of the streptomycete types were associated in common with the endpoint, control, and overhead ELF sites. Although there was some variation between the summer to fall samples, the predominant types were present at both sampling times (indicating a fairly stable population). As the predominant type (number 2) in the nursery system (Table 17) was not found on the ELF study sites and has a very distinctive red color on SCA, this type can conveniently be used to monitor changes in streptomycete populations when nursery red pine are planted on the ELF study sites in 1984.

APPENDIX A

Description of Streptomyces Types

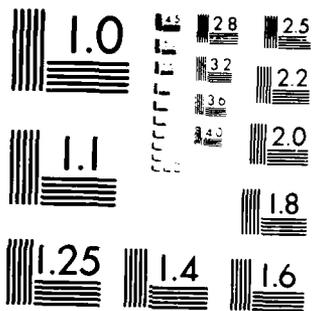
APPENDIX B

Tests with Calcium Oxalate Agar Media Concerning
Actinomycete Strain Capability to Utilize an
Oxalate Carbon Source

TESTS CONDUCTED WITH CALCIUM OXALATE AGAR MEDIA

Extensive studies with calcium oxalate agar media (COA) were initiated in the summer of 1983. Summer and fall samples were spread-plated on COA plates as well as on starch casein agar (SCA) plates. As shown in Table 1-4, little difference was found between the viable counts of heterotrophic bacteria (HB) and streptomycetes on SCA and COA. Comparing our results to a previous study which found that 50 percent of all colonies produced a clearing of COA indicating utilization of oxalate as a carbon source (Knutson et al. 1980), no clearing was observed on our COA spread-plates. However, Knutson et al. (1980) also found that approximately equal numbers of HB including streptomycetes grew on COA as on the general purpose nutrient agar. The extensive growth observed on COA may have obscured clearing of COA in our early studies. The amount of growth was probably due to 1) carry-over of nutrients from the soil or root samples and/or 2) use of yeast extract in the COA medium as an alternative carbon and energy source. To test these possibilities, COA was prepared with and without yeast extract using sterile toothpicks in a manner eliminating carry-over of agar from the richer primary isolation medium. Cultures of HB (including streptomycetes) originally isolated on COA containing yeast extract were transferred to gridded COA plates lacking yeast extract. Fourteen of the 35 cultures grew on COA without yeast extract, indicating that these organisms utilized oxalate as a carbon source. However, no clearing of COA was noted during these tests either.

During the ensuing process of identifying streptomycetes isolated from summer and fall collections, four streptomycete cultures (one each isolated from birch and pine roots at the overhead antenna site, one from birch roots at the second control site, and one from pine roots at the first control site) were found which utilized oxalate as a carbon source in COA lacking



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

yeast extract and also degraded calcium oxalate crystals (leaving a clear zone beneath and surrounding the colonies). Eleven other streptomycete isolates grew on COA lacking yeast extract but did not clear the medium. Results of streptomycete characterization are presented in Appendix A. As a result of tests conducted in 1983, samples collected in 1984 will be spread-plated on both COA lacking yeast extract and SCA for primary isolation purposes.

Table B1. Comparison of viable counts of heterotrophic bacteria and streptomycetes isolated on starch casein and calcium oxalate media from red pine rhizosphere soil and washed mycorrhizae (Summer 1983).

Sample Location	Date Collected	Viable Counts ^A										
		-----Starch Casein Agar-----					----- Calcium Oxalate Agar -----					
		Soil		Washed Roots		Soil		Washed Roots		Soil		Washed Roots
Heterotrophic Bacteria ^B	Streptomycetes	Heterotrophic Bacteria	Streptomycetes	Heterotrophic Bacteria	Streptomycetes	Heterotrophic Bacteria	Streptomycetes	Heterotrophic Bacteria	Streptomycetes	Heterotrophic Bacteria	Streptomycetes	
Overhead	7/18	2.52x10 ⁶	9.23x10 ⁴					2.29x10 ⁶				
Control	7/19	6.76x10 ⁶	1.94x10 ⁵					8.86x10 ⁶				
Control	7/19			1.21x10 ⁵	3.57x10 ⁴							
Endpoint	7/18	2.82x10 ⁷	9.2x10 ⁵					2.48x10 ⁷	7.36x10 ⁵	1.76x10 ⁶	1.37x10 ³	
Endpoint	7/18			1.85x10 ⁶	1.38x10 ⁵					8.58x10 ⁵	6.92x10 ³	
Endpoint	7/18			8.29x10 ⁵	1.23x10 ⁴					6.74x10 ⁵	6.82x10 ²	

^A Reported per g dry weight sample and mean of two replica plates.

^B Includes numbers of streptomycetes.

Table B2. Comparison of viable counts of heterotrophic bacteria and streptomycetes isolated from washed birch mycorrhizae onto starch casein agar and calcium oxalate agar media (Summer 1983).

Sample Location	Date Collected	Viable Counts ^A			
		-----Starch Casein Agar-----		--- Calcium Oxalate Agar ---	
		Washed Roots		Washed Roots	
		Heterotrophic Bacteria ^B	Streptomycetes	Heterotrophic Bacteria	Streptomycetes
Overhead	6/22	7.31x10 ⁷	1.96x10 ⁵	7.31x10 ⁷	1.96x10 ⁵
Control (second)	7/20	3.05x10 ⁷	1.78x10 ⁶	1.90x10 ⁶	
Control (first)	6/23	5.35x10 ⁷	1.96x10 ⁶	5.04x10 ⁷	
Control (first)	6/23	4.14x10 ⁷	2.14x10 ⁶	3.51x10 ⁷	

^A Reported per g dry weight sample and mean of two replica plates.

^B Includes numbers of streptomycetes.

Table B3. Comparison of viable counts of heterotrophic bacteria and streptomycetes isolated on starch casein agar and calcium oxalate agar media from birch rhizosphere soil (Fall, 1983).

Sample Tree	Sample Location	Date Collected	Viable Counts ^A			
			-----Starch Casein Agar-----		--- Calcium Oxalate Agar ---	
			Soil		Soil	
			Heterotrophic Bacteria ^B	Streptomycetes	Heterotrophic Bacteria	Streptomycetes
Birch-2	Overhead	10/10	8.28x10 ⁶	5.32x10 ⁵	6.10x10 ⁶	7.6x10 ⁵
Birch-3	Overhead	10/10	1.75x10 ⁷	2.68x10 ⁶	1.07x10 ⁷	
Birch-4	Overhead	10/10	1.33x10 ⁷	1.18x10 ⁷	2.20x10 ⁶	
Birch-8	Control (second)	10/10	4.95x10 ⁶	1.02x10 ⁶	3.81x10 ⁶	
Birch-9	Control (second)	10/10	5.31x10 ⁵	1.18x10 ⁵	5.90x10 ⁵	
Birch-10	Control (second)	10/10	7.30x10 ⁷	1.22x10 ⁵	8.50x10 ⁶	

^A Reported per g dry weight sample and mean of two replica plates.

^B Includes numbers of streptomycetes.

Table B4. Comparison of viable counts of heterotrophic bacteria and streptomycetes isolated on starch casein agar and calcium oxalate agar media from red pine rhizosphere soil (Fall, 1983).

Sample Tree	Sample Location	Date Collected	Viable Counts ^A			
			-----Starch Casein Agar-----		--- Calcium Oxalate Agar ---	
			Heterotrophic Bacteria ^B	Streptomycetes	Heterotrophic Bacteria	Streptomycetes
Pine-1	Endpoint	10/10	7.58x10 ⁶	1.43x10 ⁶	6.10x10 ⁶	7.60x10 ⁵
Pine-2	Endpoint	10/10	1.18x10 ⁷	3.57x10 ⁶	3.50x10 ⁶	7.00x10 ⁵
Pine-3	Endpoint	10/10	1.13x10 ⁷	1.88x10 ⁶	5.99x10 ⁶	8.60x10 ⁵
Pine-4	Overhead	10/10	3.40x10 ⁷	7.08x10 ⁵	8.90x10 ⁶	
Pine-5	Overhead	10/10	2.10x10 ⁷	7.00x10 ⁵	1.61x10 ⁷	
Pine-6	Overhead	10/10	2.03x10 ⁷	8.76x10 ⁵	2.92x10 ⁶	
Pine-10	Control (second)	10/10	4.46x10 ⁶	8.25x10 ⁴	6.60x10 ⁶	
Pine-11	Control (second)	10/10	1.05x10 ⁷	2.58x10 ⁵	3.87x10 ⁶	6.45x10 ⁵
Pine-12	Control (second)	10/10	3.04x10 ⁶	3.30x10 ⁵	6.60x10 ⁵	

^A Reported per g dry weight sample and mean of two replica plates.

^B Includes numbers of streptomycetes.

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"ELF Communications System Ecological Monitoring Program"
The Effects of Exposing the Slime Mold Physarum polycephalum
to Electromagnetic Fields

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GLOSSARY - ACRONYMS

- Respiration:** A measurement of the rate of oxygen utilization.
- Antenna ground:** A conducting connection between the transmitting antenna and the earth.
- Axenic culture:** Growth of a single organism (slime mold) in the absence of contaminating organisms such as bacteria, fungi, etc.
- Plasmodium:** A mass of protoplasm visible to the eye containing numerous nuclei; the entire structure is delimited by a plasma membrane. In the laboratory it is usually maintained on a solid substrate such as agar or filter paper.
- Micro-plasmodia:** Plasmodia maintained in submerged shake flasks.
- Shake flask cultures:** A method of maintaining plasmodia in a liquid nutrient medium. The flask is continuously shaken to provide oxygen to the culture.
- Cell cycle:** The number of hours between successive divisions of a cell; in this experiment it is the number of hours required for division of the nucleus.

INTRODUCTION

We have previously shown that continuous laboratory exposure of the slime mold Physarum polycephalum to weak, extremely low frequency electromagnetic fields similar to those associated with the ELF communication system can depress the rate of respiration and lengthen the mitotic cell cycle (Goodman et al. 1976, 1979). We now seek to determine whether similar effects occur when the mold is exposed to fields generated by the communication antenna itself.

The research program undertaken to answer this question contains both a laboratory and field component. Since our earlier work only involved continuous EMF exposure, we first had to determine whether intermittent exposure was capable of altering the cell's physiology. These data were needed for two reasons: first, the antenna is currently on an intermittent duty cycle rather than a continuous 24 hr/day cycle; and second, if carefully controlled intermittent laboratory exposure proved incapable of inducing significant effects, then it is unlikely that studies in an "uncontrolled" field environment would show any perturbations. The specific program questions posed were:

1. Does intermittent laboratory exposure to electromagnetic fields affect the cell cycle and respiration rate of Physarum polycephalum?
2. Does field exposure under and near the ELF communication antenna affect either the respiration rate or the cell cycle of Physarum polycephalum?

As described in the last report, attainment of these objectives requires that we divide the program into several defineable tasks. Although some tasks have already been completed, a brief description is included to provide the reader with a complete overview of our program.

ELEMENT I: Selection of Control and Experimental Sites.

Three sites (1 control, 2 experimental) have been selected. One "exposure" site is located in the vicinity of the ground (Site 7G3, see Map - Appendix I) and the other "exposure" site is located above a buried section of the antenna (Site 7A-1). The control

site is 10 miles east of the buried antenna (Site 7C-1) at a point where the ambient fields are about 2 orders of magnitude less intense than at the exposure sites. Measurements of the field intensities at the selected sites by IITRI in 1983 matched well with measurements made in 1982 (Appendix J).

ELEMENTS 2 AND 7: Development of Protocols for Field Exposure and Maintenance of Physarum polycephalum.

Field Exposure System: The transition from the laboratory implies more than giving up tight environmental controls; it also means giving up tight control of the experimental exposure conditions. As in the case of temperatures, one must substitute monitoring, of the variation in electric and magnetic fields to which the cultures are exposed, both through direct measurements on a regular cycle and through such indirect means as the WTF log of operations. The laboratory exposure regimens to which the field conditions are compared are therefore only approximations - as close approximations as one can make, but still approximations - of the exposures in nature.

The field exposure system being used has undergone significant revisions over that originally described (first Annual Report, November, 1982). Cultures are now maintained in autoclavable, polyethylene growth chambers (7"x7"x2 1/4") with reasonably tight fitting lids; carbon electrodes placed 6" apart and about 1/4" from the bottom transect each chamber. The electrodes are used to propagate electric fields. Each growth chamber was placed inside another polyethylene carrier chamber (10"x10"x12") with a tight fitting lid to provide a water proof environment for the cultures. During the experimental period 2-3 growth chambers will be placed in the outer carriers to provide multiple exposure systems at each site. Two electrical contacts transected the carrier container about 6" from the bottom. A 1/2" U-shaped vent pipe with cotton at both ends was attached to the lid for gas exchange. The outer container with the growth chamber inside was placed at the appropriate site (two separate exposure systems/site) in a hole about 20"x20"x20". At each end of the hole (in line with the predominant electric field)

an 8" square copper collecting plate was buried. Wire leads were attached from the collecting plates to a plug on the outer wall of the carrier chamber; another set of leads ran from a plug inside the carrier to the carbon electrodes of the growth chamber. To protect the system from direct sunlight, foraging animals, etc. each hole was covered with a plywood board and overlain with a 2" layer of dirt.

Although field measurements were not performed on a routine basis in 1983, protocols were developed to measure the ground E fields, the total current through the culture (measurements were made before and after subculturing without disturbing the culture), and the E fields and resistance at the collector plates. Although continuous temperature monitoring was deleted from our original protocols, the temperature in the pits were periodically recorded at the time cultures were transferred. The data showed that the temperatures on a given day were within 0.2 C. In the 1984 season, continuous temperature monitoring will be performed at each site.

In the absence of ferromagnetic materials, the magnetic field that one measures near the culture represents the field to which it is being subjected. In contrast, the situation for the electric field is considerably more complicated. In a laboratory environment, if the copper current collectors are in contact with a voltage gradient of 0.7 V/m, a separation of 20 inches will give about three times the applied field with a separation of about seven inches. However, the situation at the test sites is considerably more complicated, and thus the choice of contact plate positioning and design was the result of a series of trade-offs.

The basic difference between applying the electric field in the lab and at the WTF is that the lab voltages are from low impedance, easily controlled, and stable sources. At the WTF tapping, the potential gradient induced in the earth results in a source of high impedance. The impedance is dependent on both the conditions of the exposure, such as the nature of the soil and rocks in which the antenna is inducing currents, and the conditions of the experiment, such as the nature of the contacts between the collector

plates and the soil. Furthermore, the impedance is variable, depending upon the moisture in the soil, corrosion on the collector plates, and a number of other factors. A reliable, relatively low impedance contact in the soil could be made using techniques routinely used to make a ground connection for a dwelling. Thus, by pounding a copper pipe ten feet or so into the earth, it would establish good contact due to both the great surface area involved and to its penetration of the water table. In designing our collectors we attempted to more closely model the situation encountered by a mold growing in the wild, where it lives in the moist leaf layer on the surface of the forest's soil. The contact between the leaf layer and the soil is dependent on moisture conditions and other variables. Further, the contact between the soil and the moist leaf layers can often be separated by distances of a foot or more, due to the presence of insulating layers of dried leaves, rocks, logs, and other materials. The moist layers would behave as conduction paths in a way not dissimilar to the wiring and agar of our test chambers.

Culture Maintenance: The field exposed cultures must be maintained in an axenic state. The growth containers were described above; plasmodia were maintained on a nutrient medium consisting of 50% growth medium (Daniel and Baldwin, 1964), 50% water, and 3% agar.

Approximately 150 ml of nutrient agar was added to each growth chamber to a depth of about 1/2". After numerous false starts and contamination problems the following transfer and maintenance protocols were developed and functioned successfully. All transfers are performed in a laminar flow hood (with a 0.3 μ M Hepa filter) and a bank of uv lights (for pre-sterilization). The carrier chamber is disconnected from the wire leads at the exposure site and brought to the mobile lab. The outside of the chamber was washed with Zorbicide (to kill molds, yeasts, bacteria, etc.) and brought into the lab where the inner container(s) were removed and washed with disinfectant. The growth chamber(s) is/are placed in the hood where a sample is subcultured. The remaining plasmodium is either used at the Wisconsin Test Facility to measure

respiration or returned to Parkside for cell cycle analysis. Because of the nature of the cell cycle measurements, plasmodium exposed on agar at the W.T.F. were returned to liquid microplasmodial culture for 24 hours prior to determining the length of their mitotic cycle. After transfer, the new chambers were placed into new carrier containers previously exposed to uv light (24 hours) at the Wisconsin Test Facility. Since each field site has two carrier chambers with 4-6 exposure containers, one is always maintained as a reserve in case of contamination. Because of extensive contamination problems at the beginning of the field studies, no culture was maintained in an axenic state for more than 21 consecutive days. In all field exposures, samples will be monitored at least weekly to ascertain whether perturbations have been induced.

ELEMENTS 3 AND 4: Establishment of a Laboratory Exposure System and Determination of the Effects of Intermittent EMF Exposure.

Physarum polycephalum was maintained as submerged shake cultures in rectangular flasks; stainless steel electrodes comprise two sides of the flask (Goodman et al., 1975). Microplasmodia were subjected to intermittent (16 hrs/day, 5 days/week) electromagnetic fields of 76 Hz, 1.0 G, 1.0 V/m.

Effects of Intermittent Exposure on Mitosis: The purpose of the laboratory simulation experiments was to provide information that would be relevant to the studies being performed in the field. Since our previous experiments only involved continuous exposure to electromagnetic fields (EMF) we had to first establish that intermittent exposure could alter the cell cycle and secondly the approximate length of exposure time required to induce an effect. Control (non-exposed) cultures were placed in one incubator and the EMF cultures were maintained in another incubator. Temperature was controlled using a master-slave arrangement previously described. To perform an experiment, cultures in the log phase of growth (24 hours after transfer) were harvested, centrifuged (250 x g), the old medium decanted, and the packed volume noted. The microplasmodia were washed in distilled water, recentrifuged as above, decanted and

resuspended in two volumes of distilled water; two ml of this suspension was inoculated to filter paper (Schleicher and Schuell, #576, 8.2 cm) supported by stainless-steel mesh grids. Cultures were consecutively numbered (1-10) and the code identifying the origin of these cells was noted. This information was not available to the person determining the onset of mitosis to insure that the cultures were scored blind.

Most reports in the literature suggest a waiting period of 60 minutes from the time microplasmodia are inoculated to filter paper until the addition of growth medium. Because of the low humidity in the laboratory, we found that the cultures left for an hour became too dried resulting in more erratic cell cycles. In April, the waiting period was reduced to 45 minutes and in August it was again reduced to 30 minutes. Examination of the data also reveals that the cell cycle time of both EMF-exposed and control cultures decreased as the waiting period before the addition of medium (17.0 ml) was decreased.

The time of medium addition was noted; both culture sets were then placed on a shelf in the control incubator. To measure cell cycle effects, the time from the addition of medium until each plasmodium reached metaphase of the second (or third) mitosis was determined. At periodic intervals, the code was broken and analyses of the data performed.

In performing experiments on the bio-effects of EMF one must always be concerned with the fact that changes can occur in the culture itself which might be misread as an effect. To address this problem we require that any perturbation be reproducible before accepting the result as an EMF effect. In all experiments, duplicate lines of both control and experimental microplasmodia are set up simultaneously; microplasmodia for a given experiment are randomly selected from each set. The rationale is based on the assumption that if a spontaneous change occurs in one culture from a set, the divergence would be readily discerned. If both cultures in a set reveal a statistically significant change from control values, one must determine whether the alteration is indeed a result of EMF exposure. We address the latter problem by attempting to replicate the effect(s)

by introducing a subset of the control culture into the field being studied. If the results prove to be consistent the effect is accepted; if the data disagree, an entirely new set of control and experimental cultures are initiated and the protocols are repeated.

Results and Discussion: In the first set of experiments, microplasmodia exposed for about 180 days showed a significant acceleration in the mitotic rate relative to non-exposed controls (E = 16.54 hrs vs. C = 17.25 hrs) (see Table 1). Since this effect is the opposite of that observed with continuous EMF-exposure, we attempted to determine whether the acceleration in the cell cycle was induced by the intermittent fields or represented an incubator difference. To address the latter possibility, the exposure systems were switched; the control incubator became the exposure incubator and vice versa. At the same time, the reproducibility of the effect was tested by setting up a new line of EMF-exposed microplasmodia that were derived from the control set.

The cell cycle data from the original exposed set, 60 days after the incubators had been switched, show that the EMF-exposed culture continue to divide at a significantly faster rate (E = 16.29 hrs vs. C = 17.03 hrs) (Table 1).

In contrast, data from the new cultures placed in the field at the time the incubators were switched show that EMF-exposed cultures divided slower than the control cultures (E = 15.4 hrs vs. C = 15.1 hrs) see Table 1. At this time, the reason for the inversion in the cell cycle data relative to the first experiment cannot be explained. The fact that the EMF-exposed culture continued to divide at a faster rate even after switching incubators indicates that we are not observing an incubator effect. It is possible that the original culture or the duplicate has undergone a change that may or may not be related to EMF exposure.

To address this question, another experiment was initiated using newly germinated sclerotia from the original plasmodial inoculum. We observed that the mitotic cycle of the new cultures (before exposure) were faster than either of the earlier sets (completing the third mitosis in about 17-18 hours); therefore, all data in the third set represent the

time to the third rather than the second mitosis. The data show that intermittent EMF exposure again lengthened the cell cycle ($E = 18.1$ hrs vs. $C = 17.1$ hrs) after less than 60 days exposure (see Table I). The longer delay observed in this experiment (about 1 hr) is a result of observing the third rather than the second mitosis; we previously showed that when EMF exposure is continuous, the mitotic delay is additive (Goodman *et al.*, 1976). It also suggests that in future experiments, we should score the third rather than the second mitosis since the significance of an effect can be established earlier.

Additional evidence that suggests the data from the last experiment does not represent an artifact was the observation that both cell cycles remained unchanged during the first month of EMF-exposure; however, with continued exposure the mitotic cycle effect became manifest (see Appendix D).

It should also be stated that first, we have presented all the data because of our belief that scientific integrity requires presentation of all data even if it disagrees with a preconceived notion of what should happen.

Secondly, reversals of sign in effects are not all that rare in the bioelectromagnetics field. In fact, the study of such reversals forms a major part of other studies underway in our laboratory with NIH support. Several recent papers (Moore, 1979; Aarholt *et al.* 1981; Toroptsev and Taranov, 1982) describe studies in which reversals of effect have been observed with only small changes in field conditions.

And finally, in the early stages of our laboratory studies there were several problems with quality control of the field exposure apparatus. These problems have since been corrected. Thus, we cannot rule out the possibility that small, even medium-sized, changes in field conditions may have occurred between studies 1/2 and 3 and 4.

Effect of EMF Exposure on the Respiration Rate: The same culture lines used to ascertain the effects of weak electromagnetic fields on mitosis were also used to study respiration. To perform a respiration measurement, microplasmodia were transferred to new growth medium at the same time flasks were set up for mitosis (24 hours prior to

beginning an experiment); this approach insured that cultures were in the log phase of their growth cycle. Two ml of aerated growth medium was added to a reaction vial of a Yellow Springs Instrument (Y.S.I.) Model 53 oxygen monitor. A one ml microplasmoidal suspension was removed from the growth flask and added to the reaction vessel; the culture was suspended with a (10 mm x 3 mm) magnetic stirring bar. At the end of the experiment, microplasmidia were removed, decolorized, and their protein content determined (Goodman *et al.*, 1979).

In the first set of experiments, no significant differences ($p < 0.01$) in the QO_2 ($\mu l O_2$ consumed/mg protein/minute) of EMF-exposed cultures were observed after 180 days of exposure. However, a possible trend toward an increased respiration rate for the EMF-exposed cultures may be evident (see Table II). Following the switch of the control and exposure incubators, the QO_2 data from the original cultures after 270 days of intermittent exposure show that EMF-exposed cultures continue to display an elevated respiration rate ($QO_2 E = .83$ vs. $QO_2 C = .76$) (see Table II).

The new plasmodial line placed in the EMF environment after the incubator switch also showed an increase in its respiration rate ($QO_2 E = .90$ vs. $QO_2 C = .83$) after more than 140 days exposure (see Table II). (NOTE: Unlike the first set, these EMF-exposed cultures showed a lengthened mitotic cycle.)

The QO_2 of the newly germinated cultures were equivalent after 60 days of exposure (see Table II).

An obvious question raised by these data is why the cell cycle and respiration rate of the control and EMF-exposed cultures is not constant. Although cultures are grown in temperature controlled incubators capable of maintaining temperature differences between incubators to within 0.3 C in a stable external environment, we noticed that the baseline temperatures in each incubator could change as much as 1 C depending on room temperature. For example, as the ambient room temperature approached the incubator set point (as occurred during seasonal transition time) the incubator temperature tended

to increase. Since the incubators are connected through a master-slave control arrangement, identical changes occurred simultaneously in both incubators.

Finally in comparing these data with earlier experiments one must exercise a degree of caution in view of the differing exposure regimens (continuous vs. intermittent). In this laboratory (see References 4-7) and in others it is becoming an established fact that waveform, intensities and length of exposure can produce very different effects.

Conclusions:

1. The mitotic cell cycle is altered following intermittent EMF exposure.
2. The cell cycle effect appears to be more sensitive to EMF exposure than the respiration rate since it requires a somewhat shorter exposure period to become manifest.
3. The respiration rate of microplasmodia exposed to intermittent EMF is elevated relative to non-exposed controls.

**ELEMENT 5: Development of Protocols for Measuring O₂ Consumption at
The Wisconsin Test Facility.**

Cultures maintained in the field are maintained on a semi-solid agar substrate. Over the past year we have been devising methods to use S-3A oxygen analyzer (Applied Electrochemistry) to perform these analyses. This instrument was selected because it can measure the decrease in the O₂ content of the gas phase with high resolution. Further, the YSI instrument is not compatible with cultures maintained on an agar surface.

Unfortunately, about six months of wasted effort was expended in attempting to calibrate the instrument. After many frustrating attempts we eventually determined that the zirconia detector was defective. Once corrected, we began the job of both calibrating the instrument and designing a minimum volume cell that would both accept the agar sample and be capable of maintaining a constant temperature. A cell is

currently being constructed and should be available for testing in about 30 days.

ELEMENT 6: Development of Computer Programs for Data Analysis.

Computer programs have been developed for 1) data entry, 2) screening, and 3) analysis. The data entry program provides direct entry of the raw data, protein standards, and any other information necessary to calculate the results of a given protocol. Screen prompts request the data as to type (mitosis, O_2) in a format that minimizes error. Entries that lie outside of a reasonable range are highlighted and the operator is requested to verify the data.

Analysis of Data: We employ two techniques for statistical analysis, a parametric paired t-test, and a non-parametric randomization test. The non-parametric test is fast and used only as an indicator of when a full non-parametric test is warranted. The non-parametric computation on the APPLE computer requires approximately one hour for a preliminary computation of 1000 trials, and overnight for a full 10,000 trials.

The parametric paired t-test is described in Appendix K. Note that if the data is all collected on a single day, the variance computed in item 2 is the "conventional" paired-t variance. Item three outlines how the variance is estimated for more than one day's data. The crucial point of course is the number of degrees of freedom assigned to the statistic when assessing its significance. This is given in item 4. Note here that we employ a conservative estimate of degrees of freedom from the point of view of being able to describe a difference as being statistically significant. This can be seen by noting that if only one control value and one experimental value are measured on each day, we would assign zero degrees of freedom to the statistic.

It must be emphasized that the parametric computation is not the one we rely on for significance of an effect. It is used only as a quick and approximate measure of the statistical significance of a difference. The randomization test is relied upon to assess the significance of an observed difference, and this test does not require that we assign degrees of freedom to the computed statistic. We have enclosed a listing of the program

we used to perform this computation together with a sample listing using hypothetical data (Appendix L). In this example, the parametric measure of significance is $p = 0.024$ and the non-parametric measure is $p = 0.034$. We regard this agreement as excellent, especially in view of the small number of data used in this example.

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TABLE I
Summary of Intermittent EMF-Exposure Effects
on Mitosis Average Length of Cell Cycle

Experiment Number	Control	EMF-Exposed	MII	MIII	Number of Days Exposed
1	*17.25	16.54	X		240
2	*17.03	16.29	X		339
3	*15.1	15.4	X		140
4	*17.13	18.15		X	30

Experiment 1: Cultures placed in control and EMF incubators.

Experiment 2: Control and EMF incubators switched, cultures maintained in their appropriate incubators.

Experiment 3: Another experimental culture started by placing a subculture of the control into the new EMF incubator.

Experiment 4: Newly germinated plasmodia introduced into control and EMF incubators.

MII: The number of hours from addition of medium until the second metaphase of mitosis.

MIII: The time from the addition of medium until the third metaphase of mitosis.

*: Difference is significant at $p < 0.01$.

TABLE II

Summary of EMF-Exposure Effects on the Respiration Rate
($\dot{V}O_2 = \mu l O_2$ consumed/mg protein/minute)

Experiment Number	$\dot{V}O_2$		Number of Days Exposed
	Control	EMF-Exposed	
1	.79	.81	> 180
2	*.76	.83	> 270
3	*.83	.90	> 140
4	.83	.85	> 60

Experiment 1: Cultures placed in control and EMF incubators.

Experiment 2: Control and EMF incubators switched, cultures continue to be maintained in the appropriate incubators.

Experiment 3: Another experimental culture started by placing a subculture from the control into the new EMF incubator.

Experiment 4: Newly germinated plasmodia introduced into the control and EMF incubators.

*: Differences are significant at $p < 0.01$.

APPENDIX A

Summary of the Effects of 76 Hz, 1.0 G, 1.0 V/m on the Mitotic Cell Cycle of Physarum polycephalum (before incubator switch)

These tables compare the onset of the second mitotic division (following addition of medium) in Control and EMF exposed (Expt'l) plasmodia. The summaries show the overall average mitotic time of the Experimental and Control cultures, the average difference and the standard deviation of the difference. This is followed by the number of data (NTOT), the Degrees of Freedom, and the T-Statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

	Expt'l	Control
Overall Average:	17.97	18.01
Average Difference:		-.04
Standard Dev. of Diff.	.083	
NTOT=	79	
Deg. Freedom=	63	
T-Statistic=	.5717	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	2-1-83	2.033*	55
2.	2-2-83	3.2882**	55
3.	2-8-83	1.7333	55
4.	2-9-83	1.1232	55
5.	2-14-83	.063	55
6.	2-16-83	.6496	55
7.	2-21-83	.6373	56
8.	2-28-83	1.8083	55

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	Expt'l	Control
Overall Average:	17.11	17.42
Average Difference:		-.31
Standard Dev. of Diff.	.08	
NTOT=	88	
Deg. Freedom=	70	
T-Statistic=	3.8059**	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	3-1-83	3.2152**	62
2.	3-7-83	3.543**	62
3.	3-8-83	2.6581**	62
4.	3-9-83	3.3575**	62
5.	3-21-83	4.819**	62
6.	3-22-83	5.3988**	63
7.	3-23-83	5.5211**	62
8.	3-28-83	1.1023	63
9.	3-29-83	3.0058**	62

	Expt'l	Control
Overall Average:	16.54	17.25
Average Difference:		-.71
Standard Dev. of Diff.	.07	
NTOT=	118	
Deg. Freedom=	94	
T-Statistic=	10.1822**	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	4-4-83	10.2197**	86
2.	4-5-83	8.6619**	87
3.	4-6-83	8.026**	86
4.	4-11-83	9.3706**	86
5.	4-12-83	10.1435**	86
6.	4-13-83	11.3481**	86
7.	4-18-83	9.4641**	86
8.	4-19-83	8.90370001**	86
9.	4-20-83	11.4747**	86
10.	4-25-83	9.8757**	86
11.	4-26-83	9.8725**	86
12.	4-27-83	9.9496**	87

APPENDIX B

Summary of the Effects of 76 Hz, 1.0 G, 1.0 V/m on the Mitotic Cell Cycle of Physarum polycephalum (after incubator switch)

These tables compare the onset of the second mitotic division (following addition of medium) in Control and EMF exposed (Expt'l) plasmodia. The summaries show the overall average mitotic time of the Expt'l and Control cultures, the average difference and the standard deviation of the difference. This is followed by the number of data (NTOT), the Degrees of Freedom, and the T-Statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.



	Expt'l	Control
Overall Average:	16.29	17.03
Average Difference:		.74
Standard Dev. of Diff.	.09	
NTOT=	39	
Deg. Freedom=	31	
T-Statistic=	8.3224**	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	6-21-83	9.72720001**	23
2.	6-22-83	8.6382**	23
3.	6-28-83	4.5332**	24
4.	7-12-83	5.9354**	23

APPENDIX C

Summary of the Effects of 76 Hz, 1.0 G, 1.0 V/m on the Mitotic Cycle of Physarum polycephalum (new line after incubator switch)

These tables compare the onset of the second mitotic division (following addition of medium) in Control and EMF exposed (Expt'l) plasmodia. The summaries show the overall average mitotic time of the Expt'l and Control cultures, the average difference and the standard deviation of the difference. This is followed by the number of data (N TOT), the Degrees of Freedom, and the T-Statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

	Expt'l	Control
Overall Average:	17.50	16.94
Average Difference:		.56
Standard Dev. of Diff.	.06	
NTOT=	69	
Deg. Freedom=	55	
T-Statistic=	9.2725**	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	6-29-83	8.44**	47
2.	7-11-83	13.4591**	48
3.	7-12-83	.3448	47
4.	7-13-83	9.16340001**	47
5.	7-18-83	9.8277**	47
6.	7-20-83	14.6254**	47
7.	7-26-83	4.4477**	47



	Expt'l	Control
Overall Average:	16.41	15.88
Average Difference:		.53
Standard Dev. of Diff.	.08	
NTOT=	76	
Deg. Freedom=	60	
T-Statistic=	6.5755**	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	8-16-83	7.3781**	52
2.	8-17-83	6.5181**	52
3.	8-22-83	5.0608**	56
4.	8-23-83	5.4841**	52
5.	8-24-83	5.6534**	52
6.	8-29-83	6.7104**	52
7.	8-30-83	5.9942**	52
8.	8-31-83	6.6242**	52

	Expt'l	Control
Overall Average:	16.04	15.80
Average Difference:		.24
Standard Dev. of Diff.	.04	
NTOT=	80	
Deg. Freedom=	64	
T-Statistic=	6.0118**	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	9-6-83	7.5799**	56
2.	9-7-83	5.8175**	56
3.	9-8-83	3.5284**	56
4.	9-20-83	2.6396*	56
5.	9-19-83	6.0757**	56
6.	9-21-83	7.5637**	56
7.	9-26-83	6.8736**	56
8.	9-27-83	4.8891**	56

	Expt'l	Control
Overall Average:	15.42	15.067
Average Difference:		.35
Standard Dev. of Diff.	.06	
NTOT=	50	
Deg. Freedom=	40	
T-Statistic=	5.6676**	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	10-4-83	4.7945**	32
2.	10-6-83	6.1177**	32
3.	10-10-83	5.5039**	32
4.	10-11-83	4.093**	32
5.	10-12-83	4.8416**	32

Compute P-Value for T-Test by Randomization.

10000 Permutations out of 1.01625502E + 12 possible found 0 permutations with greater T-statistics.

P = 0 for T-Test by randomization.

APPENDIX D

Summary of the Effects of 76 Hz, 1.0 G, 1.0 V/m on the Mitotic Cell Cycle of Physarum polycephalum (new line from germinated sclerotia)

These tables compare the onset of the second mitotic division (following addition of medium) in Control and EMF exposed (Expt'l) plasmodia. The summaries show the overall average mitotic time of the Expt'l and Control cultures, the average difference and the standard deviation of the difference. This is followed by the number of data (N1, N2), the Degrees of Freedom, and the T-Statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

	Expt'l	Control
Overall Average:	17.49	17.47
Average Difference:		.02
Standard Dev. of Diff.	.05	
NTOT=	80	
Deg. Freedom=	64	
T-Statistic=	.4615	

*P .05

**P .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	11-14-83	1.0571	56
2.	11-15-83	.7307	56
3.	11-16-83	.1539	56
4.	11-21-83	.3997	56
5.	11-22-83	.3983	56
6.	11-28-83	2.363*	56
7.	11-29-83	.5656	56
8.	11-30-83	.3323	56

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	Expt'l	Control
Overall Average:	18.15	17.13
Average Difference:		1.02
Standard Dev. of Diff.	.05	
NTOT=	79	
Deg. Freedom=	63	
T-Statistic=	20.281**	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	12-6-83	18.0755**	55
2.	12-7-83	20.2678**	55
3.	12-12-83	22.0568**	55
4.	12-13-83	19.8782	55
5.	12-14-83	19.3947**	55
6.	12-19-83	18.4673**	56
7.	12-20-83	15.4681**	55
8.	12-21-83	18.8118**	55

Compute P-Value for T-Test by Randomization

10000 permutations out of $8.1315686E+18$ possible.

NG = 0 after 244 permutations and P = 0.

NG = 0 after 6868 permutations and P = 0.

NG = 0 after 6870 permutations and P = 0.

APPENDIX E

Summary of Effects of 76 Hz, 1.0 G, 1.0 V/m on the QO_2

(μ l O_2 consumed mg protein/min)

of Physarum polycephalum

(before incubator switch)

These tables compare the oxygen consumption in Control and EMF-exposed (Expt'l) microplasmodia. The summaries show the overall average QO_2 for Expt'l and Control cultures, the average difference and the standard deviation of the difference. This is followed by the number of data (NTOT), the Degrees of Freedom, and the T-Statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

	Expt'l	Control
Overall Average:	.74	.74
Average Difference:		6.0
Standard Dev. of Diff.	.009	
NTOT=	58	
Deg. Freedom=	44	
T-Statistic=	.0703	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	1-11-83	1.384	40
2.	1-12-83	.8095	38
3.	1-13-83	.6698	38
4.	1-20-83	1.8485	34
5.	1-25-83	2.2759*	38
6.	1-26-83	.9388	38
7.	1-27-83	2.4974*	38

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	Expt'l	Control
Overall Average:	.72	.74
Average Difference:		-.02
Standard Dev. of Diff.	.01	
NTOT=	56	
Deg. Freedom=	42	
T-Statistic=	1.4313	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	2-1-83	1.1825	36
2.	2-2-83	.7765	36
3.	2-3-83	2.7372**	36
4.	2-8-83	.0991	36
5.	2-9-83	.8118	36
6.	2-10-83	3.0937**	36
7.	2-15-83	.6482	36

	Exp'l	Control
Overall Average:	.81	.80
Average Difference:		.01
Standard Dev. of Diff.	.008	
NTOT=	62	
Deg. Freedom=	44	
T-Statistic=	1.5098	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	3-15-83	2.1198*	38
2.	3-16-83	1.3419	38
3.	3-17-83	2.827**	38
4.	3-22-83	.41	38
5.	3-23-83	2.2981*	40
6.	3-24-83	1.7909	40
7.	3-29-83	.564	40
8.	3-30-83	1.8191	40
9.	3-31-83	.7631	40

	Expt'l	Control
Overall Average:	.91	.88
Average Difference:		.03
Standard Dev. of Diff.	.009	
NTOT=	58	
Deg. Freedom=	38	
T-Statistic=	2.3846*	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

Remove Day	T-Statistic	Degrees of Freedom
1. 4-5-83	2.4187*	34
2. 4-7-83	2.1653*	34
3. 4-12-83	2.2434*	34
4. 4-13-83	2.8937**	34
5. 4-14-83	1.7448	34
6. 4-19-83	2.4172*	34
7. 4-20-83	2.5105*	36
8. 4-21-83	1.7046	34
9. 4-26-83	1.8356	34
10. 4-27-83	2.7654**	34

APPENDIX F

Summary of Effects of 76 Hz, 1.0 G, 1.0 V/m on the QO_2 (μ l O_2 consumed mg protein/min) of Physarum polycephalum (after incubator switch)

These tables compare the oxygen consumption in Control and EMF-exposed (Expt'l) microplasmodia. The summaries show the overall average QO_2 for Expt'l and Control cultures, the average difference and the standard deviation of the difference. This is followed by the number of data (NTOT), the Degrees of Freedom, and the T-Statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

	Expt'l	Control
Overall Average:	.93	.94
Average Difference:		-.01
Standard Dev. of Diff.	.009	
NTOT=	46	
Deg. Freedom=	30	
T-Statistic=	.5162	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	5-3-83	2.3816*	26
2.	5-4-83	1.14	26
3.	5-5-83	1.4793	26
4.	5-12-83	.471	26
5.	5-17-83	2.2322*	28
6.	5-19-83	.5647	26
7.	5-25-83	.9503	26
8.	5-26-83	.4211	26

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	Exptl	Control
Overall Average:	.83	.76
Average Difference:		.05
Standard Dev. of Diff.	.006	
NTOT=	60	
Deg. Freedom=	40	
T-Statistic=	10.2555**	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

Remove Day	T-Statistic	Degrees of Freedom
1. 6-7-83	9.8645**	36
2. 6-8-83	6.1171**	36
3. 6-9-83	10.6286**	36
4. 6-14-83	8.8669**	36
5. 6-15-83	9.1801**	36
6. 6-16-83	10.796**	36
7. 6-21-83	8.6307**	36
8. 6-22-83	12.459**	36
9. 6-28-83	9.6754**	36
10. 6-30-83	11.0484**	36

APPENDIX G

Summary of Effects of 76 Hz, 1.0 G, 1.0 V/m on the $\dot{Q}O_2$ ($\mu l O_2$ consumed mg protein/min) of Physarum polycephalum (new line after incubator switch)

These tables compare the oxygen consumption in Control and EMF-exposed (Expt'I) microplasmidia. The summaries show the overall average $\dot{Q}O_2$ for Expt'I and Control cultures, the average difference and the standard deviation of the difference. This is followed by the number of data (NTOT), the Degrees of Freedom, and the T-Statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

	Expt'l		Control
Overall Average:	.79		.81
Average Difference:		-.01	
Standard Dev. of Diff.	.006		
NTOT=	46		
Deg. Freedom=	30		
T-Statistic=	1.9436		

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	7-12-83	.0891	26
2.	7-13-83	5.9137**	26
3.	7-14-83	2.1686*	26
4.	7-19-83	.1111	26
5.	7-21-83	2.7722*	26
6.	7-26-83	.8992	26
7.	7-27-83	1.2477	26



	Expt'l	Control
Overall Average:	.84	.82
Average Difference:		.02
Standard Dev. of Diff.	.008	
NTOT=	54	
Deg. Freedom=	36	
T-Statistic=	2.5246*	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	8-2-83	1.994*	32
2.	8-13-83	2.9986**	32
3.	8-14-83	4.2295**	32
4.	8-16-83	1.5549	32
5.	8-17-83	.9588	32
6.	8-23-83	2.979**	32
7.	8-24-83	2.5927**	32
8.	8-30-83	2.6724**	32
9.	8-31-83	1.7689	32

	Expt'l	Control
Overall Average:	.9	.82
Average Difference:		.08
Standard Dev. of Diff.	.007	
NTOT=	50	
Deg. Freedom=	32	
T-Statistic=	9.8266**	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	9-1-83	6.7982**	28
2.	9-7-83	7.6976**	28
3.	9-8-83	11.257**	28
4.	9-20-83	10.7491**	28
5.	9-21-83	9.7956**	28
6.	9-22-83	10.9757**	28
7.	9-27-83	9.1251**	28
8.	9-28-83	8.5957**	28
9.	9-29-83	8.8275**	32

	Expt'l	Control
Overall Average:	.85	.78
Average Difference:		.07
Standard Dev. of Diff.	.01	
NTOT=	18	
Deg. Freedom=	12	
T-Statistic=	5.1686**	

*P . .05

**P . .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	10-5-83	5.0919**	8
2.	10-6-83	2.8033*	8
3.	10-7-83	4.5696	8

APPENDIX H

Summary of Effects of 76 Hz, 1.0 G, 1.0 V/m on the QO_2 (QO_2 consumed mg protein/min) of Physarum polycephalum (new line from germinated sclerotia)

These tables compare the oxygen consumption in Control and EMF-exposed (Expt'l) microplasmodia. The summaries show the overall average QO_2 for Expt'l and Control cultures, the average difference and the standard deviation of the difference. This is followed by the number of data (NTOT), the Degrees of Freedom, and the T-Statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

	Expt'l	Control
Overall Average:	.79	.82
Average Difference:		-.03
Standard Dev. of Diff.	.02	
NTOT=	42	
Deg. Freedom=	28	
T-Statistic=	1.2841	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	11-15-83	1.0721	24
2.	11-16-83	.6478	24
3.	11-17-83	.3743	24
4.	11-22-83	3.2421**	24
5.	11-23-83	1.5316	24
6.	11-29-83	1.0863	24
7.	11-30-83	.9576	24

	Expt'l	Control
Overall Average:	.82	.80
Average Difference:		.02
Standard Dev. of Diff.	.01	
NTOT=	44	
Deg. Freedom=	28	
T-Statistic=	.4675	

*P < .05

**P < .01

Recompute T-Statistic Removing One Day at a Time

	Remove Day	T-Statistic	Degrees of Freedom
1.	12-1-83	.6815	24
2.	12-7-83	.5002	24
3.	12-7-83	.3825	28
4.	12-13-83	.2519	24
5.	12-14-83	1.111	24
6.	12-15-83	.6468	24
7.	12-20-83	.3672	24
8.	12-21-83	2.7263*	24

APPENDIX I

Map of control (7C1), ground (7G3), and buried antenna (7A1) sites located in the Chequamegon Forest at the Wisconsin Test Facility.

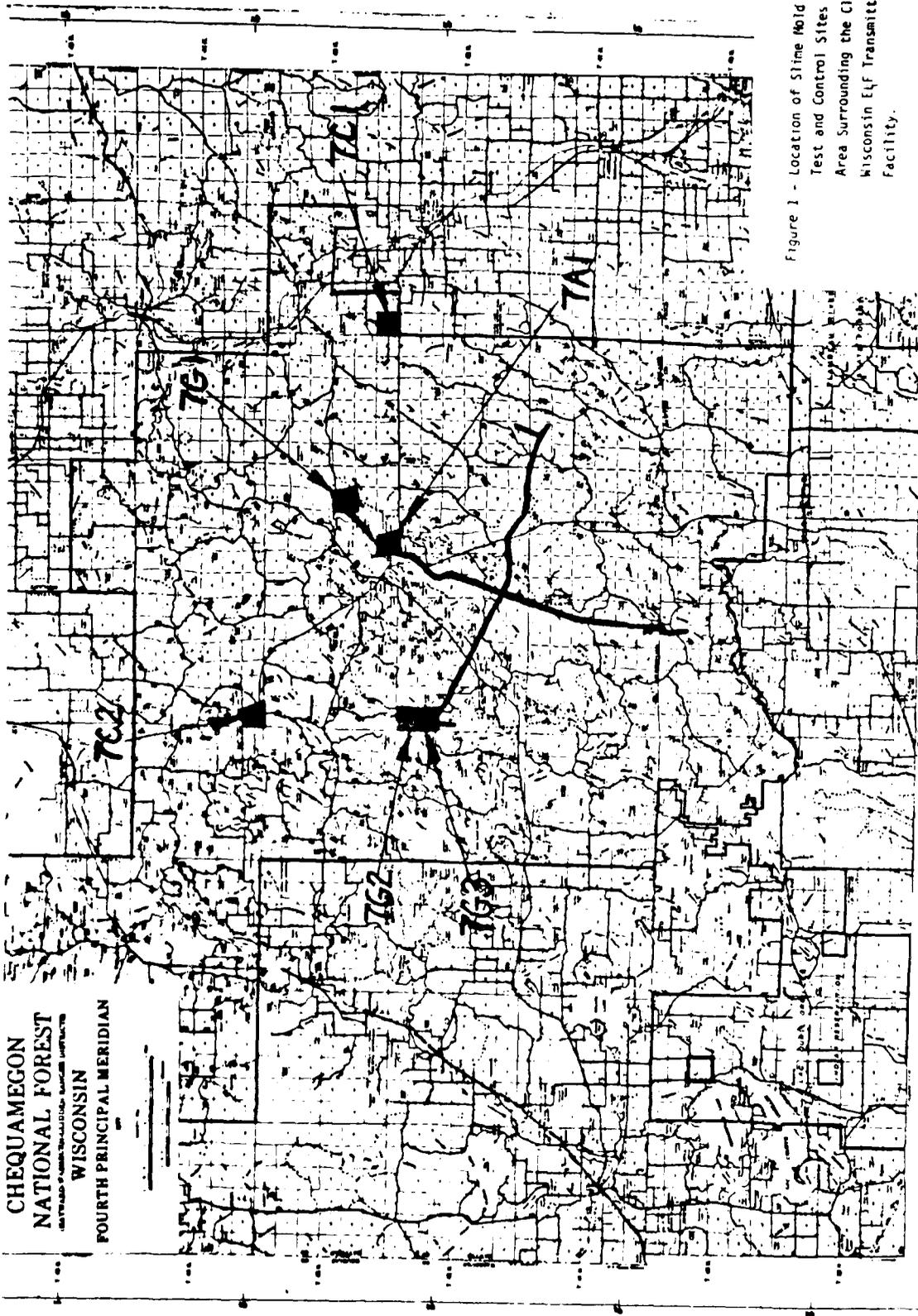


Figure 1 - Location of Slime Mold Study Test and Control Sites in the Area Surrounding the Clam Lake, Wisconsin ELF Transmitter Facility.

APPENDIX J

Electric and magnetic field intensities measured by IITRI at the control (7C1), ground (7G3), and buried antenna sites (7A1).

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TABLE 2
 =====
 ELECTROMAGNETIC FIELD INTENSITIES
 AND FLUX DENSITIES (1)

SITE NO.	MEAS PT	TRANSVERSE ELECTRIC FIELD (IN THE AIR) INTENSITY (V/m)		LONGITUDINAL ELECTRIC FIELD (IN THE EARTH) INTENSITY (V/m)		MAGNETIC FLUX DENSITY (Gauss)	
		76 HZ	60 HZ	76 HZ	60 HZ	76 HZ	60 HZ
7A1	1	A	A	0.17	0.00013	0.15	0.000019
7G3	1	"	"	1.9	0.000091	0.0053	0.000001
7C1	1	"	"	0.0019	0.000070	0.000025	<0.000001

1) Values shown are magnitudes determined as the square root of the sum of the squares of the orthogonal field components measured. Data for 76 Hz represent worst case values determined by summation of the magnitudes of the fields produced by the E-W and N-S antennas extrapolated to full operating current (300 Amps).

A) Data not taken

APPENDIX K

Method for computation of paired T-test.

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METHOD FOR COMPUTATION OF PAIRED T-TEST

1. The best estimate of the mean square variability for a given day, i , is obtained by pooling variation in control and experimental observations. Thus,

$$MS_i = \frac{SS_{ci} + SS_{Ei}}{(\eta_{ci} - 1)(\eta_{Ei} - 1)} \equiv \frac{SS_i}{N_i - 2}$$

Note: $SS_i \equiv SS_{ci} + SS_{Ei}$

$$N_i \equiv \eta_{ci} + \eta_{Ei}$$

2. The variability for Δ_i on a given day is obtained by adding estimated variance for control and experimental cultures from that day:

$$\sigma = \frac{MS_i}{\eta_{ci}} + \frac{MS_i}{\eta_{Ei}} = MS_i \left(\frac{\eta_i}{\eta_{ci} \cdot \eta_{Ei}} \right) = \frac{N_i SS_i}{(N_i - 2)(\eta_{ci} \eta_{Ei})}$$

By definition, then

$$MS_i \equiv N_i \sigma_i^2 = \frac{N_i^2 SS_i}{(N_i^2)(\eta_{ci} \cdot \eta_{Ei})}$$

3. The estimate for the variance of the overall difference, $\bar{\Delta}$, is obtained by computing a weighted average of the daily mean square variations,

$$MS_{TOT} = \frac{\sum (N_i - 2) MS_i}{\sum (N_i - 2)}$$

$$\sigma_{TOT}^2 = \frac{MS_{TOT}}{\sum N_i} = \frac{\sum N_i^2 SS_i / (\eta_{ci} \cdot \eta_{Ei})}{\sum N_i \sum (N_i - 2)}$$

4. The t-statistic with $(\sum N_i - 2 \times \text{number of days})$ degrees of freedom is

$$t = \bar{\Delta} / \sigma_{TOT}$$

Symbol Definitions:

SS_{Ci} = sum of squares for control data for day i

SS_{Ei} = sum of squares for experimental data for day i

n_{Ci} = number of control data on day i

n_{Ei} = number of experimental data on day i

N_i = total number of data for day i

Δ = average difference between control and experimental for day i

$\bar{\Delta}$ = overall average difference for all days

APPENDIX L

A listing of the program used to perform statistical analysis of mitosis data using hypothetical data.

Example: STATISTICAL ANALYSIS
OF MITOSIS DATA USING
HYPOTHETICAL DATA

4/84

Parametric Computation

MITOSIS DATA ANALYSIS FOR DATA TYPE = M2

DAY/ (DIFF.)	EXPT'L.	AVERAGE	CONTROL	AVERAGE
DAY ONE ** (ZERO=6)	15 (9) 15.5 (9.3) 16 (10) 15 (9)	[15.4]	14 (8) 14.5 (8.3) 15 (9) 15 (9)	[14.8]
	[.6]		15.5 (9.3)	
DAY TWO ** (ZERO=6.3)	15 (9.3) 14.5 (9) 14.25 (8.45) 15.5 (10)	[14.81]	13 (7.3) 14.5 (9) 13.5 (8) 14.5 (9)	[14.05]
	[1.7625]		14.75 (9.15)	

Start @ 6:30pm
1st Control @ 7:30am
1st Exptl @ 9:30am

OVERALL AVERAGE: 15.1063
AVERAGE DIFFERENCE: .6812 hr
STANDARD DEV. OF DIFF. .2718

NTOT= 19
DEG. FREEDOM= 15
T-STATISTIC= 2.5068*

* P<.05 ** P<.01

← 4.7% diff E > C

actual value of P = 0.024

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
1. DAY ONE	1.6754	7
2. DAY TWO	1.8974	8

check for Robustness of parametric computation. This would not be considered a robust result. Note, however, the n is small.

NON-PARAMETRIC COMPUTATION

COMPUTE P-VALUE FOR T-TEST BY RANDOMIZATION
1000 PERMUTATIONS OUT OF 31752 POSSIBLE
NG=3 AFTER 153 PERMUTATIONS AND P=.0196078431
NG=11 AFTER 294 PERMUTATIONS AND P=.037414966
NG=13 AFTER 412 PERMUTATIONS AND P=.0315533981
NG=20 AFTER 569 PERMUTATIONS AND P=.0351493849
NG=23 AFTER 705 PERMUTATIONS AND P=.0326241135
NG=33 AFTER 942 PERMUTATIONS AND P=.0350318471
FOUND 34 PERMUTATIONS WITH GREATER T-STATISTICS

P=.034 FOR T-TEST BY RANDOMIZATION

INTERMEDIATE Readouts requested by keyboard query during computation

← Note reasonable agreement with parametric computation P=.034 vs P=.024

LISTING of "MITOSIS"
4/84

```

1 REM MITOSIS ANALYSIS BY PAIRED-T TEST/ M. T. MARRON 4/83
2 REM
3 REM RANDOM FUNCTION SEED CORRECTED 12/83; M2/M3 MODIFICATION 3/84
4 DEF FN R2(X) = INT (X * 100 + 0.5) / 100
5 DEF FN R4(X) = INT (X * 10000 + 0.5) / 10000
6 TIME = RND (- RND (1)); PRINT CHR$(4)*"BRUN USER#": REM LOAD USER# + RANDOM FUNCTION
7 DIM D$(30),NN(30,2),O(30,15),E(30,15),CC(30),EE(30),DL(30),T5(31),T1(31),FA(12),ZERO(30),CM(30,15),EM(30,15)
8 DATA 1,2,6,24,120,720,5040,40320,362880,3,6288E6,3,99,68E7,4,796016E8
9 FOR I = 1 TO 12: READ FA(I): NEXT
10 CIE = 1:IOS = 0: REM SELECT PRINTER, NO=0 AND YES=1
11 DATA 12,706,63,657,4,303,9,925,3,132,5,841,2,776,4,604,2,571,4,032,2,447,3,707,2,365,3,499,2,306,3,355,2,262,3,250,2,220,3,169
12 DATA 2,201,3,106,2,179,3,055,2,16,3,012,2,145,2,977,2,191,2,947,2,12,2,921,2,11,2,895,2,101,2,878,2,093,2,861,2,086,2,845
13 DATA 2,08,2,831,2,074,2,819,2,069,2,807,2,064,2,797,2,060,2,787,2,056,2,779,2,052,2,771,2,048,2,763,2,045,2,756,2,042,2,750,1,96,2,
58
14 FOR I = 1 TO 31: READ T5(I),T1(I): NEXT
15 TEXT : HOME : PRINT "MITOSIS ANALYSIS": PRINT : GOSUB 70: PRINT
16 GOTO 100
17 REM *****
18 REM * SUBROUTINES
19 REM *****
20 FOR I = 1 TO NMAX
22 IF I = 0 THEN PRINT " ": GOTO 26
24 PRINT C(KDAY,I)
26 IF I = NE THEN PRINT " ": GOTO 30
28 PRINT E(KDAY,I)
30 NEXT I
32 RETURN
36 FOR K9 = 1 TO 4: FOR K8 = 1 TO 10: K7 = PEEK (49200): NEXT K8: NEXT K9: RETURN : REM RASPBERRY
38 OK = 1: FLAGNEG = 0: MINFLAG = 0: RETURN : REM RESET FLAGS FOR DECIMAL CHECK
40 OK = 0: REM CHECK D$(KDAY) FOR NON-NUMERIC CHARACTER
42 FOR I2 = 1 TO LEN (D$(KDAY))
44 B = ASC (MID$(D$(KDAY),I2,1))
46 IF (B = 58 AND B > 47) OR (B = 46) THEN 50
48 OK = 1: RETURN
50 NEXT I2
52 PRINT "DATE MUST CONTAIN 1 OR MORE": PRINT " ALPHABETIC CHARACTERS": RETURN
60 REM SUBROUTINE CONVERTS HR:MIN DATA TO DECIMAL FORMAT AND CHECKS
61 REM FOR INSTANCES OF NEGATIVE DATA AND MIN VALUES <80. NO ERROR FLAG
62 REM SETTINGS ARE OK=1, MINFLAG=0, AND FLAGNEG=0. -9 IS VALUE IN & OUT.
63 IF X9 < 0 THEN OK = 0: FLAGNEG = 1
64 I9 = INT (X9): X8 = (X9 - I9) / 0.6
65 IF X8 > = .99 THEN OK = 0: MINFLAG = 1
66 X9 = I9 + X8: RETURN
70 REM INPUT ANALYSIS TYPE, M2 OR M3?
71 INPUT "ANALYSIS OF M2 OR M3?": TYPE$
72 IF TYPE$ < > "M2" AND TYPE$ < > "M3" THEN GOSUB 36: PRINT : PRINT "INPUT EITHER M2 OR M3
": GOTO 71
73 RETURN
74 REM ADJUST TIME DIFFERENCES TO ACCOUNT FOR 12 HR PERIODS
75 X9 = 12 + X9 - 2 + (X9 - 2 < 0) * 12 + (TYPE$ = "M3") * 12
76 IF TYPE$ = "M2" THEN RETURN
77 IF X9 < 19 THEN X9 = X9 + 12
78 IF X9 > 29 THEN X9 = X9 - 12
79 RETURN
80 PRINT D$*PRN1$: PRINT CHR$(9): CHR$(17): RETURN : REM TURN PRINTER ON AND CHANGE GRAPPLER CONTROL TO CTRL-G
90 PRINT D$*PRN0$: RETURN : REM TURN PRINTER OFF
95 PRINT I$:: FOR I = 1 TO L9: PRINT " ": NEXT I: PRINT : RETURN
96 PRINT I$:: FOR I = 1 TO L8: PRINT " ": NEXT I: PRINT : RETURN

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96 PRINT I$; FOR I = 1 TO L8: PRINT "-"; NEXT I: PRINT : RETURN
100 REM *****
102 REM * PAIRED GROUPS T-TEST
104 REM *****
105 C$ = CHR$(27):D$ = CHR$(4):I$ = CHR$(9)
106 KDAY = 0:DD = 0:CAVE = 0:EAWE = 0
107 KDAY = KDAY + 1
108 PRINT : INPUT "DATE:";D$;KDAY/
109 IF LEN(D$;KDAY) = 0 THEN GOTO 400: REM FINISH DATA INPUT
110 GOSUB 40: IF OK = 0 THEN 115
111 EE KDAY = 0:CC(KDAY) = 0
112 REM *****
113 REM * ENTER DATA
114 REM *****
115 REM IN TIME CONVERSIONS FROM HR:MIN TO DECIMAL FORMAT IT HAS BEEN ASSUMED THAT ZERO TIME IS BETWEEN 2 AND 7 PM AND MIDNIGHT IS 18
    BEYOND THE NEXT DAY AFTER 7AM.
116 OK = 1: INPUT "ENTER ZERO TIME "A9:Z2 = X9: GOSUB 63: IF OK THEN 140
117 GOSUB 36: PRINT "PLEASE RE"; GOTO 134
118 Z = 1:DEFO KDAY = Z: GOSUB 38:NC = 0: PRINT "ENTER CONTROL DATA"
119 INPUT C$: IF LEN(C$) = 0 THEN GOTO 150
120 NC = NC + 1:A9 = VAL(C$;CM(KDAY,NC)) = X9: GOSUB 63: GOSUB 75:D(KDAY,NC) = X9: GOTO 142
121 PRINT "CONTROL TIMES:"; FOR I = 1 TO NC: PRINT " "CM(KDAY,I); NEXT I
122 IF NOT (OK) THEN PRINT "SOMETHING WRONG HERE"
123 IF MINFLAG THEN PRINT "AT LEAST ONE ENTRY HAS MINUTES >60"
124 IF FLAGNEG THEN PRINT "AT LEAST ONE ENTRY IS LESS THAN 0"
125 INPUT "DO YOU WISH TO RE-ENTER SOME DATA?";A$
126 IF LEFT$(A$,1) = "Y" GOTO 120
127 GOSUB 38: INPUT "CHANGE WHICH ENTRY?";IC: IF IC < 0 OR IC > 1 THEN GOSUB 36: GOTO 160
128 INPUT "NEW VALUE=";C$: IF LEN(C$) = 0 THEN 162
129 X9 = VAL(C$;CM(KDAY,IC)) = X9: GOSUB 63: GOSUB 75:D(KDAY,IC) = X9: GOTO 150
130 REM
131 GOSUB 39:MM(KDAY,1) = NC:NE = 0: PRINT "ENTER EXPERIMENTAL DATA"
132 INPUT C$: IF LEN(C$) = 0 THEN GOTO 180
133 NE = NE + 1:A9 = VAL(C$;EM(KDAY,NE)) = X9: GOSUB 63: GOSUB 75:E(KDAY,NE) = X9: GOTO 172
134 PRINT "EXPERIMENTAL TIMES:"; FOR I = 1 TO NE: PRINT " "EM(KDAY,I); NEXT I
135 IF NOT (OK) THEN PRINT "SOMETHING WRONG HERE"
136 IF MINFLAG THEN PRINT "AT LEAST ONE ENTRY HAS MINUTES >60"
137 IF FLAGNEG THEN PRINT "AT LEAST ONE ENTRY IS LESS THAN 0"
138 INPUT "DO YOU WISH TO RE-ENTER SOME DATA?";A$
139 IF LEFT$(A$,1) = "Y" GOTO 200
140 GOSUB 38: INPUT "CHANGE WHICH ENTRY?";IC: IF IC < NE OR IC > 1 THEN GOSUB 36: GOTO 190
141 INPUT "NEW VALUE=";C$: IF LEN(C$) = 0 THEN 192
142 X9 = VAL(C$;EM(KDAY,IC)) = X9: GOSUB 63: GOSUB 75:E(KDAY,IC) = X9: GOTO 180
143 MM(KDAY,2) = NE
144 REM END OF DAY'S DATA
145 FOR I = 1 TO NC:CC(KDAY) = CC(KDAY) + CM(KDAY,I): NEXT I:CC(KDAY) = CC(KDAY) / NC
146 FOR I = 1 TO NE:EE(KDAY) = EE(KDAY) + E(KDAY,I): NEXT I:EE(KDAY) = EE(KDAY) / NE
147 CC(KDAY) = EE(KDAY) - CC(KDAY):DD = DD + CC(KDAY):CAVE = CAVE + CC(KDAY):EAWE = EAWE + EE(KDAY)
148 GOTO 110
149 REM *****
150 REM * COMPUTE STATISTIC
151 REM *****
152 KDAY = KDAY - 1:NT = 0:NT = 0
153 FOR ID = 1 TO KDAY
154 S1 = 0
155 FOR I = 1 TO MM(ID,1):S1 = S1 + CC(ID,I) - CC(ID) / 2: NEXT I
156 FOR I = 1 TO MM(ID,2):S1 = S1 + EE(ID,I) - EE(ID) / 2: NEXT I
157 NT = MM(ID,1) + MM(ID,2):NT = NT + NT
158 S2 = S2 + NT * NT * S1 / MM(ID,1) * MM(ID,2)
159 NEXT ID
160 DF = NT - 2 * KDAY:SIGMA2 = S2 / (NT * DF)
161 TD = CC(KDAY):EAWE = EAWE + KDAY:EAWE = EAWE + KDAY
162 T = ABS(TD) / SQR(SIGMA2)
163 REM *****

```



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1000 FOR I = 1 TO NN(ID,2):S1 = S1 + (E(ID,I) - EE(ID)) ^ 2: NEXT
1010 N1 = NN(ID,1) + NN(ID,2):NT = NT + N1
1020 S2 = S2 + N1 * N1 * S1 / (NN(ID,1) * NN(ID,2))
1030 S3 = S3 + N1:S4 = S4 + N1 - 2
1040 DD = DD + EE(ID) - CC(ID)
1050 NEXT ID
1060 DD = DD / KM1:DF = NT - 2 * KM1:SIGMA2 = S2 / (NT * DF)
1070 ST$ = "":T = ABS (DD / SQR (SIGMA2)): IF DF > 30 THEN 1110
1080 IF T > T5(DF) THEN ST$ = "*"
1090 IF T > T1(DF) THEN ST$ = "***"
1100 GOTO 1130
1110 IF T > T5(31) THEN ST$ = "*"
1120 IF T > T1(31) THEN ST$ = "***"
1130 PRINT I$;IM;"  ":DY$(IM);I$:"  ":FN R4(T);ST$:I$:"  ":DF
1140 NEXT IM
1150 GOSUB 95: GOSUB 90
1200 REM *****
1210 REM *NONPARAMETRIC COMPUTATION
1220 REM *****
1230 PRINT : PRINT "PERFORM NONPARAM COMPUTATION (Y/N)?": GET A$: PRINT A$: PRINT
1240 IF A$ = "N" THEN END
1250 NUMBER = 1
1260 FOR ID = 1 TO KDAY
1270 N1 = NN(ID,1):N2 = NN(ID,2):N = N1 + N2: IF N > 12 THEN PRINT "THERE ARE TOO MANY DATA FOR DAY ":ID: PRINT : END
1280 NUMBER = NUMBER * FA(N) / (FA(N1) * FA(N2))
1290 NEXT ID
1310 PRINT "THERE ARE ":NUMBER:" PERMUTATIONS POSSIBLE"
1320 INPUT "HOW MANY DO YOU WISH TO PERFORM?":NPERM
1330 IF NPERM < = NUMBER THEN 1345
1340 PRINT "TOO MANY PERMUTATIONS REQUESTED": GOTO 1310
1345 NG = 0: REM # OF T'S)T-TEST
1350 GOSUB 80: PRINT : PRINT :L8 = 45: GOSUB 96: PRINT I$:"COMPUTE P-VALUE FOR T-TEST BY RANDOMIZATION": GOSUB 96: PRINT I$:NPERM:" PER
MUTATIONS OUT OF ":NUMBER:" POSSIBLE": GOSUB 90
1355 PRINT : PRINT "THIS MAY TAKE A WHILE": PRINT "1  ";
1360 FOR IP = 1 TO NPERM: REM PERMUTATION LOOP
1370 PRINT ".":S2 = 0:S3 = 0:DD = 0
1380 FOR ID = 1 TO KDAY: REM DAY LOOP
1390 S1 = 0:N1 = NN(ID,1):N2 = NN(ID,2):N = N1 + N2:CAVE = 0:EAVE = 0
1400 FOR I = 1 TO N1:K = USR (N2) + 1:TEMP = C(ID,I):C(ID,I) = E(ID,K):E(ID,K) = TEMP: NEXT
1410 FOR I = 1 TO N2:K = USR (N1) + 1:TEMP = E(ID,I):E(ID,I) = C(ID,K):C(ID,K) = TEMP:EAVE = EAVE + E(ID,I): NEXT
1420 FOR I = 1 TO N1:CAVE = CAVE + C(ID,I): NEXT
1430 CAVE = CAVE / N1:EAVE = EAVE / N2:DD = DD + EAVE - CAVE
1440 FOR I = 1 TO N1:S1 = S1 + (C(ID,I) - CAVE) ^ 2: NEXT
1450 FOR I = 1 TO N2:S1 = S1 + (E(ID,I) - EAVE) ^ 2: NEXT
1460 S2 = S2 + N * N * S1 / (N1 * N2)
1465 NEXT ID: REM END DAY LOOP
1470 TTEMP = DD / SQR (S2): IF ABS (TTEMP) < TH0D THEN 1480
1475 NG = NG + 1
1480 IF (IP - INT (IP / 20) * 20) = 0 THEN PRINT : PRINT IP:"  ";
1485 IF PEEK (49152) < 128 THEN 1495
1490 POKE 49168,0: PRINT
1492 GOSUB 80: PRINT I$:"NG=":NG:" AFTER ":IP:" PERMUTATIONS AND P=":NG : IP: GOSUB 90
1495 NEXT IP: REM END PERM LOOP
1500 PRINT : PRINT "NGREATER=":NG:" FRACTION=":NG / NPERM
1505 GOSUB 80: PRINT I$:"FOUND ":NG:" PERMUTATIONS WITH GREATER T-STATISTICS"
1510 LP = 31: GOSUB 95: PRINT I$:"P=":NG / NPERM:" FOR T-TEST BY RANDOMIZATION": GOSUB 95: GOSUB 90

```

1. Cover Page:

a. Subcontractor's name and address:

Rudolph Neal Band
Department of Zoology
Michigan State University
East Lansing, Michigan 48824

b. Subcontract number: E06516-82-C-20015

c. Title: ELF communications System Ecological Monitoring
Program, Task 5.2, Soil Amoeba.

d. Reporting year: November 8, 1982 to November 7, 1983.

2. Frontispages:

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b. Subcontract number: E06516-82-C-20015

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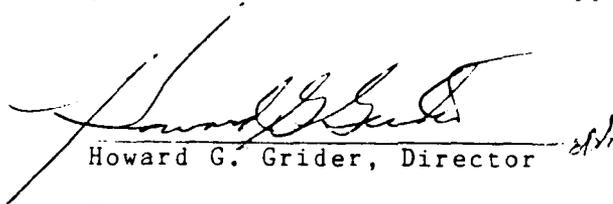
d. Reporting year: November 8, 1982 to November 7, 1983.

e. Name and signature of principal investigator:


Rudolph Neal Band, PI

f. Co-investigators: none

g. Name and signature of subcontractor's approving and releasing
authority:


Howard G. Grider, Director
Contract and Grant Admin.

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4. Abstract:

Experimental and control study sites were identified in the 1983 field season. Sites were characterized as to electromagnetic background, physical and chemical properties, and biological characteristics. Some of this work will continue into the 1984 season. This is in marked contrast to the 1982 field season when study sites were not known.

Studies of soil amoebae were designed to provide sufficient data to determine sample sizes and methods of statistical analysis suitable for comparing control and experimental sites. Studies of species diversity are in progress.

5. Summary:

Amoebae are common soil organisms present in large numbers (i.e. ca. 4,000/g soil). Ecologically their role in soil formation from organic matter (i.e. mineralization) is to eat bacteria and fungi. They are also capable of preying on multicellular organisms including man, although this is not common. In turn, amoebae are eaten by others, notably fungi and bacteria. A gram of soil typically contains at least three species of amoebae (which add up to 4,000 individuals) so that they are doing different things together in a small soil unit.

As cells, they are used extensively in Cell Biology in place of or in conjunction with cells from animals including man. Examples can be seen in cancer research, studies of muscle contraction, developmental biology and molecular biology. This is possible because their basic structure and function is the same as liver cells, heart cells, or any other cell from higher animals. Consequently any physiological effects induced by ELF on cells of higher organism would also affect soil amoebae.

Any ecological stress induced by electromagnetic effects of ELF would be reflected in detectable changes in the soil amoeba population. Changes would include numbers of active amoebae, species diversity, their physiology and their distribution in soil. Consequently, the activity of amoebae in soil away from the antenna will be compared with amoebae from similar soils near the antenna and ground wire.

In the 1983 field season, control and experimental sites were identified which resembled each other biologically, physically and chemically. In addition the final sites were monitored for ambient electric and magnetic effects in soil by IITRI. Criteria for electromagnetic exposure were established by IITRI and will be discussed later. Background electromagnetic radiation was found acceptable for control, antenna and ground wire sites. Sampling and growth experiments were done to determine variability and statistical methods appropriate for comparing control and experimental sites.

6. Progress report:

INTRODUCTION

Soil amoebae play a significant role in soil mineralization, acting as micro-predators and serving as food for other soil organisms. Since several soil amoebae are used in cell and molecular biology, much of the biochemical and physiological mechanisms underlying their biology is known. Thus, possible electromagnetic (EMF) effects on animal cells would be readily detected in these organisms.

possible stress induced by EMF might act directly on the organism. Goodman, E.M., Greenbaum, B., Marron, M.T. (1976. Rad. Res. 66, 431-40) published evidence of an effect on the length of the interphase portion of the cell cycle and cytoplasmic streaming of the slime mold Physarum, an amoeboid organism. Friend, Jr., A.W., Finch, E.D., Schwan, H.P. (1975. Science 187, 357-59) found that the freshwater amoeba Chaos chaos oriented to an EMF. Thus, EMF's may exert effects on basic cell functions. An EMF could also exhibit indirect effects by acting on other soil components important to the amoebae. The National Research Council (1977. Report of the Committee on Biosphere Effects of Extremely-Low-Frequency Radiation) recommended that studies on amoebae be followed up and that a general ecological study be done on the effects of EMF of extremely low frequency (ELF). The present research is part of that effort

Environmental changes in soil that take place over time (e.g. moisture, temperature, etc.) are important to this study. Electromagnetic effects might be restricted to a biological state induced by a particular environment (e.g. vegetative vs. dormant amoebae). Obviously environmental changes will directly effect soil amoebae as well. Consequently two controls are used in this study. Experimental sites (antenna and ground wire) are compared to a control site, located 9 miles south of the antenna, and all sites are studied over the growing season. In addition, sufficient data will be accumulated by the end of the next growing season to form a base line to compare to sites after the antenna is operational.

OBJECTIVES: For the 1983 field season, experimental and control study sites were identified. Sites were characterized as to electromagnetic background, physical and chemical properties. Biological studies are in progress. Field studies were designed to provide sufficient data to determine sample sizes and methods of statistical analyses suitable for comparing control and experimental sites.

3.

WORK PLAN

The following was submitted to IITRI with the first monthly progress report (July, 1982):

Period: July 1, 1982 to October 31, 1983 (actual dates are July 8, 1982 to November 7, 1983).

Element 1 - Research Facility. Synopsis: rent an on-site research facility, obtain equipment and supplies needed to perform research.

Element 2 - Plot selection and characterization. Synopsis: survey area to identify study soils, near proposed locations for the antenna and the ground wire, and a control area away from both.

Element 3 - Ambient monitoring system. Synopsis: calibrate temperature and moisture probes for the study area soils, install and use during the 1983 growing season.

Element 4 - Species and strain characterization. Synopsis: using morphological and physiological markers, identify species and strains of soil amobae from the study area.

4.

Element 5 - Biological diversity and activity. Synopsis: during the growing season, determine ratio of vegetative to dormant amoebae as it relates to ambient monitoring and to spatial distribution. Growth rates and cropping activity will be determined.

Element 6 - Data monitoring. Synopsis: When enough data is obtained, it will be analyzed statistically for possible correlations to environmental variables and to other biological data.

SCHEDULE:

Elements 1 and 2 were to have been done in the 1982 field season. Elements 3,4 and 6 were to be done over the 1982/83 season while Element 5 was to be done during the 1983 field season. Actually site selection took place during the 1983 season while, in general, the other work elements were on schedule, at a preliminary level.

EXPERIMENTAL

Methods and results will be presented in reference to the Work Plan, given above. Each element will be compared with the 1982 field work. A final section will identify planned changes, although these will be discussed in the applicable work element as well.

1. Research facility. A research facility was rented in 1982 and used through the 1983 season. Equipment ordered in 1982 was available for the 1983 field season.

2. Plot selection and characterization. In the 1982 field season, location of the antenna was unknown. Once the antenna location was known (in 1983), exploratory sites used in 1982 were too close to the antenna for use as controls and too far away for use as experimental sites. Much of the 1983 field season was devoted to plot selection. The antenna location in Dickinson County was surveyed before the 1983 field season and the portion of interest to me was not changed during. The ground wire site that was comparable to possible antenna and control sites (Ground Site No. 4) was not surveyed until the end of the field season. For the 1983 field season, I picked a probably ground wire location to study; this will be referred to as the old ground wire site while the other will be called the new ground wire site. I assume that the location of the new ground wire site will be firm by the start of the 1984 field season so that it can be characterized and used at that time.

6.

With the aid of foresters from Michigan's Department of Natural Resources (DNR), I selected several possible antenna, ground and control sites. As the study sites were identified, including the new ground wire site, this information was passed back to the DNR so that they could issue a land use permit. The selection criteria at this level included access, forest type (northern hardwood community, dominant sugar maple) and proximity to logging operations. Although a USDA soil survey is in progress, it had not been done for areas of interest to me. I used a 1938 soil survey to supplement the above criteria, although hardwood forests by their nature will be located in "similar" soils. The USDA Soil Survey personnel sampled my study sites as they were identified during the 1983 season, however the results are not available as yet.

In June and July, 1983, IIT Research Institute field crews made electromagnetic (EMF) measurements at the various sites I had selected. They found antenna, ground wire and control sites (one each) that complied with background EMF criteria. Briefly the criteria focus on electric and magnetic fields in earth rather than air. Control and experimental sites must not differ in background EMF by more than one order of magnitude; the control site must be one or two orders of magnitude less than experimental sites in regards to EMF produced by the extremely low frequency (ELF) antenna and ground wire.

7.

The control site is located approximately 9 miles south of the antenna's southern terminus at the ground wire site. The antenna study site is approximately 3.5 miles north of the ground wire site. These correspond to IITRI's site numbers 6T2, 6T3 and 6C2. I used these sites for the balance of the season, they will be referred to as antenna, ground and control sites. The new ground wire site is located slightly east of the old ground wire site and will have to be surveyed for EMF background in 1984.

Rectangular study areas, 10 X 20 m. were established at each study site. Once the antenna is operational these areas may have to be modified to provide for an area that includes EMF isobars of adequate strength. This is particularly true of the new ground wire site since it is anticipated that induced voltages from the ground wire will be quite localized. Obviously direct EMF measurements by IITRI will be needed when the antenna is operational.

Random samples were taken from each study site by using a numbered grid system, 1 sq.m., and a random number generator. Sampling was done with a 3/4" tube sampler (Oakfield model L with S-2 tip). Samples were divided into organic and mineral horizons while the sample was in the coring tube. Samples were stored in paired, plastic bags unless pooled where noted. The soil profile is typical of northern hardwood soils with a sharp difference between the organic and mineral horizons. In a soil core the 1 to 2 inch thick organic horizon is taken as a total sample while the top 2 inches of the underlying mineral horizon is used as the mineral sample.

Soil chemistry, performed by Michigan State University's Soil Testing Laboratory, was done on organic and mineral horizons from 20 pooled samples for each site. The data from the antenna site, the old ground wire site and the control site is summarized in Table 1. Small differences exist between sites but these are probably due to varying proportions of mixing between the horizons. At each site there are large differences between horizons as expected. Soil chemistry will be very useful for year to year analyses as an experimental control to detect unexpected changes at a site, especially those due to human activities. All of the study sites will be located in 1984 so that replicate determinations of soil chemistry will be done each season.

Bulk density (i.e. dry weight per cubic centimeter) measurements were done on 4 samples from each site, subdivided into organic and mineral horizons. The results are given in Table 2. One cc of the mineral horizon weighs 1g while one cc of the organic horizon weighs 0.36g, a ratio of roughly 2.9 to 1. Therefore a 1 gram sample from the organic horizon is almost three times bulkier than 1 gram of the mineral horizon. It is anticipated that #/g amoebae will be more for the organic horizon than the mineral horizon for this reason alone.

Soil pH was measured at each site over a period of 14 days. The data presented in Table 3, a one-way analysis of variance, fails to indicate a significance difference between sites and horizons.

The acidic pH observed is expected for a northern hardwood forest with a sharply stratified organic layer referred to as Mor humus.

Vegetation was a part of the initial selection criteria since dominant sugar maple, hardwood plots were selected as study sites. None of the plots included pioneer species indicative of clearing activities such as logging. All of the study sites were on high ground so the shrubs and herbaceous plants were similar. In a 20 x 20 m plot (which included the 10 X 20 m study area) at each site, the density and species of mature trees was remarkably similar:

Control site - 29 sugar maple, 1 basswood; antenna site -28 sugar maple, 8 basswood; old ground site - 32 sugar maple, 1 basswood. All sites contained small-sized elm, ash and leatherwood.

In summary, plot selection has been accomplished if the new ground wire site's background EMF fulfills the criteria established by IITRI. Soil chemistry will be repeated twice each season to monitor for unexpected changes in soil properties, especially those due to human activity. A third replicate may be needed if significant variation exists between samples. Vegetation at each site will be monitored for changes, especially the appearance of pioneer species which would be indicative of a marked change in plant cover due to tree cutting. Both the antenna and ground wire right of ways will be cleared. Study sites are located so as to be outside of the cleared areas but as close as possible to them; the direct measurement of EMF isobars at each location will be needed once the antenna is operational.

3. Ambient monitoring. Soil moisture and temperature at each site was monitored for part of the 1983 field season. Equipment necessary to do this was ordered in 1982 and available before the 1983 field season but study sites were not settled until mid-season. Data loggers from Omnidata were used to monitor soil moisture (in bars suction) and temperature (deg.C) at 1 hr. intervals. Shorter and longer monitoring intervals are possible with the instrumentation. Regardless of the monitoring interval chosen, the data logger actually determines soil moisture and temperature every 5 min. and records these at the chosen time interval as an average reading. Thus the 1 hr. reading is actually an average reading for the 1 hr. interval. If a 4 hr. interval had been chosen, the actual reading would not have been a measurement at that time but an average of the 5 min. readings over the 4 hr. interval. The data logger records the measurements into an EPROM chip which can be read into the lab micro-computer for analysis and storage. The EPROM chip itself is erased with ultra-violet light for re-use. An example of the raw data from the EPROM chip is given in Figure 1. Computer software is used to plot the data at longer time intervals without the averaging effect of the data logger. Inspection of raw data indicated that 4 hr. plots retained high and low temperature readings (e.g. Figure 2) and for trends, once daily average plots are useful (e.g. Figure 3) since the temperature spread over a 24 hr. interval is not great.

Soil temperature readings (accurate to the nearest 0.5 deg.C) were similar between sites (e.g. Figure 4) but, as expected, they were buffered in the mineral layer (e.g. Figure 2), as opposed to the upper, organic layer. The overlapping of temperatures between sites reaffirms site similarity, particularly in regards to cover vegetation.

Soil moisture is expressed in bars suction by the data loggers, the loggers are measuring the conductivity of a clay block whose moisture is in equilibrium with the surrounding soil. The blocks do suffer an equilibrium lag phase when they are saturated during a heavy rain but they are the only method available for automated monitoring of soil moisture. Soil moisture expressed in bars suction, obtained with clay block conductivity or by direct measurement of soil suction, has more biological meaning than % water. Correlations between soil suction and the size of water-filled pores have been used to study the moisture requirements of a variety of living organisms. Darbyshire (1975 in Soil Microbiology (ed. N.Walker) Wiley) did this for Colpoda. I find (unpublished) that small amoebae grow best at 0.3 bar suction and not at all at 0.5 bar, where they are found as dormant cysts. The actual moisture content of soil at a given soil suction (e.g. 0.3 bar) will vary depending on the water binding properties of the soil and its bulk density so that water content in itself is not very meaningful. The organic layer of the sites at 0.3 bars suction has a moisture content of around 60% water while the sandy, mineral layer below the same

organic layer is approximately 20% water. At 0.3 bar both horizons will support vegetative growth of amoebae. Amoebae could not be vegetative at 45% water in the organic layer or at 10% water in the mineral layer since these both represent a soil suction of 1 bar.

The soil suction data obtained by the data loggers (e.g. Figure 1,2,3-c,d) are useful to show general seasonal trends. Note that there was a relative dry spell at the beginning of September in the organic layer while the mineral layer was too dry to support significant growth of amoebae for much of September. Later in this report I will return to this issue when it is necessary to determine the ratio of vegetative amoebae to cysts in a given sample, where a more precise determination of soil suction is desirable. Anticipating this, I will use a portable soil probe that can be brought into rapid equilibrium with soil

4. Species and strain characterization. Soil samples taken in the 1982 season were enriched on low nutrient-agar and non-nutrient agar plates with Escherichia coli as the added food. The same morphological species of amoebae were enriched with either agar but there was a greater tendency for flagellate blooms in the low nutrient agar. Further, counts of amoebae in soil were the same using either form of agar. Consequently, for the 1983 season non-nutrient agar, described below was used with E. coli (strain K-12) as the added food organism. I found it much easier to isolate amoebae from the soil dilution count wells

used to enumerate amoebae in soil (see sect. #5 below) rather than from direct soil slurries. Soil slurries contained a mixture of amoebae together with other organisms. Soil dilution wells tended to contain less of a variety of organisms by virtue of the dilution procedure. For the 1983 season I am using soil dilution wells to isolate amoebae. The isolations are currently in progress, as part of the off-season analysis of soil. This makes a good sequence since soil dilution counts are made during the field season and then the plates are stored for off-season isolation and characterization of amoebae.

Samples taken from 2 sites during the 1982 field season and extensively used during the 1982/83 off-season failed to reveal species of amoebae unique to a site or to a particular horizon, based on morphology. Aside from expected species (Acanthamoeba castellanii, A. polyphaga, Naegleria gruberi (at least two morphologically distinct strains), Vahlkampfia sp., Mayorella sp., together with flagellates and Colpoda sp., two interesting species were found. A. astronyxis is a very large species which might be thought of as a freshwater organism by virtue of its size, yet it was isolated from both sites (on high ground). A new amoeboid-flagellate was isolated routinely, it will be published as a new genus and species called Kalavalia balamuthi. It is not proper for me to refer to this organism by name until I publish it, but, it is convenient since I have used it in work element #5 for growth experiments and this report is not a published paper.

Isolates from the 1983 field season are in progress. So far nothing new can be added except that another isolate of A. astronyxis has been isolated from all sites that is small for the species, it is in the size range of A. catellanii. The vegetative amoeba resembles A. castellanii morphologically but the cyst exhibits the unmistakable morphology of A. astronyxis.

The isolation of mitochondrial DNA and the study of DNA restriction fragments are in progress. This technique will focus on Naegleria sp. Toward this end isolates of Naegleria are being carefully maintained. As soon as I have cleaned up the small strain of A. astronyxis I hope that Dr. T. Byers at Ohio State will include it in his taxonomic study of Acanthamoeba mitochondrial DNA. By next year I will have enough data to describe any species diversity that may exist within Naegleria sp. at the study sites. This will enable me to observe any changes in diversity after the antenna is operational. Other markers that will be used in conjunction with the study of mitochondrial DNA restriction fragments include isozymes, similar to the work of Daggett, P. & Nerad, T.A. (1983. Protozool. 30, 126-8)

Note that much of this work element is in progress. The isolates are from work done during the 1983 field season while this work element is designed to do during the off-season.

5. Biological diversity and activity. Since study sites were not identified in the 1982 field season, little work was done on this section. In the 1983 field season most of the effort involved site selection. Portions of this section were done as part of site selection and the development of statistical procedures. This section involves different questions that require population counts of amoebae; I will discuss the counting procedure first.

The method of counting amoebae in soil samples (Singh, B.N. 1946. *Ann. Appl. Biol.* 33, 112-19; Darbyshire et al. 1974. *Rev. Ecol. Biol. Soil* 11, 465-75.) was used. Statistically this is a more powerful technique than the most probable number (MPN) method (e.g. Koch, A.L. 1981 pp.179-207 in Gerhardt, P. et al.(ed.) *Manual of methods for general bacteriology*). The MPN method, originally proposed by H.R. Halvorson in 1932 differs from Singh's application of the Fisher & Yates dilution method in principal, in practice and in power. The trade-off to the more laborious approach of Singh is a more powerful technique. Fisher derived his dilution table with a mathematical solution to a series while the MPN method is statistically based although both are ultimately based on a Poisson distribution. Note that Darbyshire et al. (1974) used both techniques and treats them separately, one for bacteria and one for amoebae. At a 95% confidence level, to illustrate the relative precision of Singh's method, two counts of 7,210 and 3,920 are not significantly different.

The statistics of Singh's method, modeled after Fisher and Yates' statistics text, establishes count limits for statistically similar counts at the 95% confidence level. However, this does not address the problem of differences between samples at a given soil site. I have addressed this problem by testing 10 replicate samples per site, each subdivided into an organic and a mineral horizon. This provides a sufficient number of degrees of freedom to do a statistical analysis.

As described in work plan element # 4 above, I initially used a low nutrient agar surface to enrich amoebae from soil, using E. coli as food. This was similar to the procedure used by Singh in that he added a coliform bacterium to his plates to enrich soil amoebae, however he used a non-nutrient agar (just NaCl). In Darbyshire's update of Singh's method, Darbyshire did not add Agar to the bottom of plastic, 96 multiwell plates--mechanically impossible when the dilution procedure is automated. In using Darbyshire's method, I used my LS-saline (Band, R.N. & Mohrlok, S. 1969. J. Gen. Microbiol. 59, 351-81) to make soil dilutions and to wash and suspend the food bacterium, E. coli (strain K12). LS-saline contains: 2.9g NaCl; 0.65g MgSO₄; 0.04g CaCl₂.

Differential counts of vegetative amoebae and cysts are done by splitting a sample in half, treating one half to kill vegetative amoebae and then counting both the untreated sample containing vegetative amoebae and cysts and the treated sample containing cysts alone. Singh killed vegetative amoebae by

soaking the soil sample in 2% HCl overnight; this was also used by Darbyshire. I use 1% sodium dodecyl sulfate (SDS), instead of 2% HCl, in LS-saline to kill vegetative amoebae. Amoebae are killed instantly by SDS while cysts are not affected by this detergent (Umeche, N. 1983. Arch. Protistenk. 127, 127-30). Soil is suspended in 1% SDS, thoroughly mixed and immediately washed several times with LS-saline by centrifugation to remove all of the SDS (essential). The short exposure to SDS may be easier on cysts of various species and it eliminates the delay in setting-up differential counts. It will also reveal inadequate mixing of soil with saline for counting. If the untreated sample is not adequately mixed, cyst clumps will not be dispersed and a lower count will be obtained for the sample that supposedly contains more amoebae (i.e. cysts and vegetative amoebae) than the treated sample that contains only cysts.

Table 4 illustrates a statistical analysis of counts obtained from 10 samples per site, split into organic and mineral horizons, and compared to a second, 10 sample count a month later (i.e. September and October). In this case a two-way analysis of variance was used on log-transformed counts to compare sites and dates for each horizon. The particular counts revealed significant differences at the 5% level for the organic horizon due to the control site which was a little high. The mineral horizons at all three sites were not significantly different at the 5% level for the two dates. Thus a significant change due to the antenna would have to be reflected by changes in both the

organic and mineral horizons. The separation of the organic and mineral horizons is critical since they differ markedly in amoeba numbers. In an analysis of variance significant differences between replicates at a given site, due to poor separation of horizons would show up in the interaction portion of an ANOVA. This is not the case for the above example. Table 4 reflects a coefficient of variation that is less than 10% of the mean for a given date. From a 90% level power curve, significant differences can be detected at 1.4 X std. dev. for a sample size of 10 while a sample size of 8 yields a figure of 1.5 to 1.6 X std. dev. A sample size of 4 only reveals a difference that exceeds 2.5 X std. dev., clearly unacceptable. Thus sample sizes of 8 and 10 are almost equally powerful.

Table 5 presents data from differential counts (i.e. vegetative vs. cyst) done in duplicate at the three sites. Obviously the data is too scattered. For example, examining the total vegetative and cyst column, the organic horizons of the control and ground sites do not differ within themselves but do differ between sites, while the antenna site organic horizon differs between samples. Next season, differential counts will have to be based on 8 or 10 samples per site as illustrated in the total count of Table 4. On the sampling day, the horizons will be separated and divided in half. One-half of each horizon will be treated immediately with 1% SDS to kill vegetative amoebae.

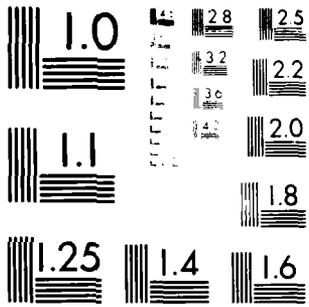
Growth experiments are to be done at the field sites. These will be done with an amoeba isolated from the sites, using E. coli as the food organism. The soil submersible culture vessel submitted to IITRI for testing consists of a small pore dialysis membrane, 44 X 44 mm with a breathing/sampling tube of sufficient length to extend above the soil surface (cotton plugged). This appears to provide adequate electrical conductivity and yet the small pore dialysis membrane does not lose water at a sufficient rate to be meaningful over the duration of the growth experiment. Counts of amoebae will be done with a haemocytometer under the microscope, in duplicate. Table 6 presents regression calculations for the growth of Kalavalia balamuthi at 13 and 15 deg.C under the above conditions except that the soils were incubated in laboratory incubators. The mean generation times (i.e. time between mitotic divisions) ranged around 8 to 9 hours. As demonstrated in Table 6, the 95% confidence intervals for the slopes at 13 and 15 deg.C overlapped. Therefore the data fails to reveal a significant difference between the three determinations of mean generation time. This procedure will be used to compare growth (i.e. time between mitotic divisions) at the three sites in the soil submersible culture vessels. Feeding efficiency (i.e. cropping activity on bacteria) will utilize the above procedure, with the added variable of using different densities of the food bacterium, E. coli.

In the above experiments fewer replicates are needed than those used for soil dilution counts because the direct counting of amoebae is more accurate.

6. Data management. Data is being accumulated on micro-computer disks using the same soft-ware program used by other terrestrial and aquatic groups studying possible effects of ELF. At any future time it would be possible to integrate this information. Although data will be manipulated on the mainframe computer, it is too costly to store data there.

Identification of planned changes:

1. Although paired plots will be used, the control plot will serve both antenna and ground sites.
2. Cluster analysis will not be done since 10 replicate samples per site did not reveal sufficient variation in amoeba numbers to indicate clustering at a macro level. Clustering at a micro level will not affect data needed to detect differences between experimental and control sites.
3. Instead of a full cell cycle analysis of an amoeba in the experimental and control sites, I propose to look at that portion of the cycle in which Dr. Goodman (1976. described earlier) described an effect of the ELF radiation on Physarum, the total interphase time between mitoses (i.e. the mean generation time). Since Acanthamoeba castellanii lacks a G1 phase (DNA replication starts as soon as the chromosomes unfold in telophase), mitosis requires 24 min. and chromosomal DNA synthesis requires 20 min.,



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

the bulk of the generation time (18 hr.) is occupied by the G2 phase (Band, R.N. and Mohrlok, S. 1973. J. Protozool. 20, 654-7). Physarum also lacks a G1 phase so that organisms are comparable.

7. Peer Reviewers:

I suggest the following as peer reviewers. I contacted them last year about this so that they are aware of my activities. I assume that IITRI will work with them directly.

1. Prof. Thomas J. Byers, Department of Microbiology, Ohio State University.
2. Prof. Fredrick L. Schuster, Department of Biology, Brooklyn College.

Table 1. Soil Chemistry:*

Element	SITE/HORIZON**					
	CO	CM	AO	AM	GO	GM
P	24	31	18	33	13	13
K	106	58	77	18	84	22
Ca	2400	800	1707	534	2628	694
Mg	146	63	123	40	165	63
Zn	72	3	31	2	37	2
Fe	16	92	8	32	8	36
Mn	227	12	180	2	195	5
Cu	3	1	2	1	4	1
Na	14	47	22	38	22	47
Cl	30	5	20	0	40	15
%Org.N	20	1.6	17	1.6	20	1.7

*Performed by Michigan State University Soil Testing Laboratory, data expressed as ppm except for %org.N.

**SITE: C, control; A, antenna; G, old ground.

HORIZON: O, organic; M, mineral.

Each site/horizon was obtained by pooling 20 random samples.

Table 2. Bulk Density (g dry wt./cc):

ORGANIC HORIZON			MINERAL HORIZON		
Antenna	Ground	Control	Antenna	Ground	Control
0.27	0.25	0.47	0.95	1.0	1.0
0.44	0.17	0.23	0.93	0.94	1.0
0.49	0.48	0.43	1.24	1.08	1.0
0.37	0.36	0.33	1.01	1.06	1.2
means;					
0.39	0.32	0.37	1.03	1.02	1.1

over all mean = $\frac{(\text{organic})}{0.3575}$
 ± 0.1082

$\frac{(\text{mineral})}{1.0342}$
 ± 0.09976

Ratio of bulk densities:

$$\text{Mineral/Organic} = 1.0342/0.3575 = 2.9$$

Table 3. Soil pH:*

SITE	HORIZON	MEAN pH \pm SD (n=14)
Control	Organic	6.54 \pm 0.37
	Mineral	6.44 \pm 0.3
Antenna	Organic	6.2 \pm 0.46
	Mineral	6.36 \pm 0.3
Ground (old)	Organic	6.52 \pm 0.33
	Mineral	6.54 \pm 0.37

One-way ANOVA:

	D.F.	M.S.
Between	5	0.2536
Within	78	0.1297
F = 1.955 (N.S.)		

* From samples taken 14 times between June 27 and Aug. 1, 1983.

Table 4. Counts from 10 samples per site, taken Sept. 7 and Oct. 16, 1983:

SITE	HORIZON	DATE	MEAN, log#/g soil ± S.D.	(MEAN) (#/g soil)
Control	Organic	9/7	3.7899 ± 0.2136	(6,165)
		10/16	3.7779 ± 0.3378	(5,997)
	Mineral	9/7	2.8952 ± 0.2806	(785)
		10/16	3.1432 ± 0.1793	(1,391)
Antenna	Organic	9/7	3.3202 ± 0.1412	(2,090)
		10/16	3.4351 ± 0.1394	(2,723)
	Mineral	9/7	3.0514 ± 0.2102	(1,126)
		10/16	3.0982 ± 0.3942	(1,254)
Ground	Organic	9/7	3.4976 ± 0.2026	(3,145)
		10/16	3.6844 ± 0.2464	(4,835)
	Mineral	9/7	2.9803 ± 0.3313	(956)
		10/16	3.1139 ± 0.0984	(1,300)

Two-way ANOVA:

		DF	MS	F
1. ORGANIC:	site	2	0.828479767	16.6409*
	date	1	0.138711214	2.7862
	interaction	2	0.050567865	1.0157
	error	54	0.049785707	--
2. MINERAL	site	2	0.087312841	0.2197
	date	1	0.015732288	4.2511
	interaction	2	0.053519773	0.7032
	error	54	0.071603303	--

* Significant difference at 5% level due to organic horizon of control site.

Table 5. Differential counts, 2 samples per site, Aug. 17, 1983:

SITE	HORIZON	TOTAL COUNT	CYST COUNT	AMOEBA NO.
Control	Organic-1	29,454	15,948	13,511
	Mineral-1	1,322	783	539
	Organic-2	14,186	7,705	6,481
	Mineral-2	2,909	1,029	1,880
Antenna	Organic-1	23,235	10,603	12,632
	Mineral-1	1,580	3,432	0
	Organic-2	4,455	3,423	1,023
	Mineral-2	8,193	2,886	5,307
Ground	Organic-1	53,715	53,715	0
	Mineral-1	6,368	2,069	4,299
	Organic-2	65,889	55,248	10,641
	Mineral-2	1,589	2,057	0

Table 6. Regression calculations for in vitro growth of Kalavalia balamuthi, log tranformed, at 13 and 15 deg.C.

Temp.	Slope	Correl. Coef. \pm SE	95%Confidence Limits for Slope
13 (4 cultures)	0.0367	0.9676 \pm 0.0034	L1 = 0.031085 L2 = 0.104502
13 (3 cultures)	0.0343	0.975 \pm 0.0015	L1 = 0.029478 L2 = 0.039039
15 (4 cultures)	0.0401	0.9787 \pm 0.002	L1 = 0.033648 L2 = 0.046486

SENSOR: 31
SITE: I
DEPTH: 1
CHIP #1

28.

DATE	TIME	SUCTION	DEGREES
9/12/83	10:00	.2	13
9/12/83	11:00	.2	13
9/12/83	12:00	.2	13.5
9/12/83	13:00	.2	13.5
9/12/83	14:00	.2	13.5
9/12/83	15:00	.2	13.5
9/12/83	16:00	.2	13.5
9/12/83	17:00	.2	13.5
9/12/83	18:00	.2	13.5
9/12/83	19:00	.2	13
9/12/83	20:00	.2	13
9/12/83	21:00	.3	13
9/12/83	22:00	.2	12.5
9/12/83	23:00	.3	12.5
9/13/83	00:00	.3	12
9/13/83	01:00	.2	12
9/13/83	02:00	.2	11.5
9/13/83	03:00	.2	11.5
9/13/83	04:00	.3	11
9/13/83	05:00	.3	11
9/13/83	06:00	.3	11
9/13/83	07:00	.3	10.5
9/13/83	08:00	.3	10.5
9/13/83	09:00	.3	10.5
9/13/83	10:00	.3	11
9/13/83	11:00	.2	11.5
9/13/83	12:00	.2	12
9/13/83	13:00	.3	12.5
9/13/83	14:00	.3	13
9/13/83	15:00	.2	13
9/13/83	16:00	.2	13
9/13/83	17:00	.3	13
9/13/83	18:00	.3	12.5
9/13/83	19:00	.3	12.5
9/13/83	20:00	.2	12.5
9/13/83	21:00	.2	12
9/13/83	22:00	.2	12
9/13/83	23:00	.3	12
9/14/83	00:00	.3	11.5
9/14/83	01:00	.3	11.5
9/14/83	02:00	.3	11
9/14/83	03:00	.3	11
9/14/83	04:00	.3	11
9/14/83	05:00	.3	10.5
9/14/83	06:00	.3	10.5
9/14/83	07:00	.3	10.5
9/14/83	08:00	.3	10.5
9/14/83	09:00	.3	10.5
9/14/83	10:00	.3	10.5
9/14/83	11:00	.3	11
9/14/83	12:00	.3	11.5
9/14/83	13:00	.2	12
9/14/83	14:00	.2	12.5

FIGURE 1. Raw data from EPROM chip taken from data logger and read on micro-computer. Data from organic layer of the control site.

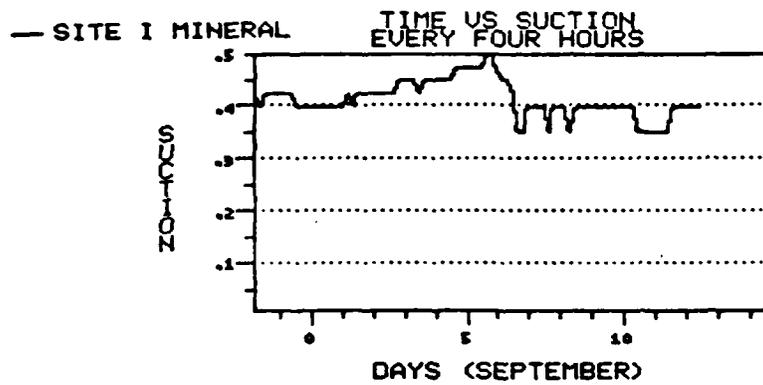
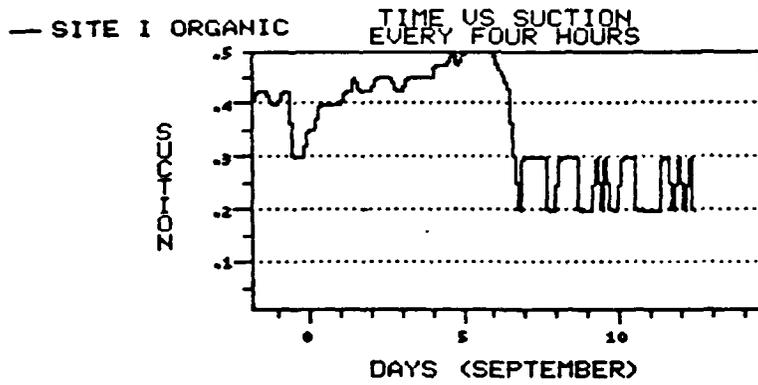
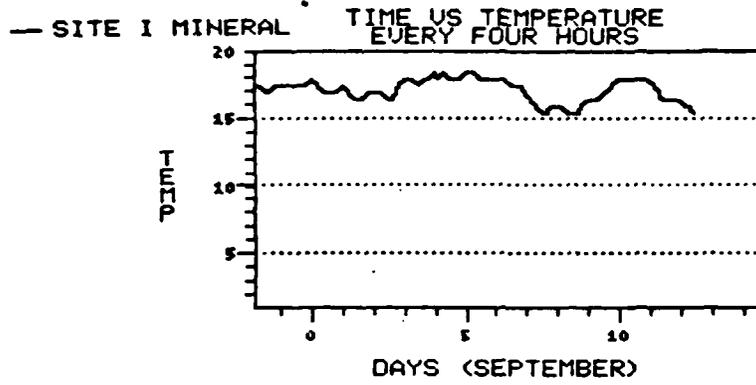
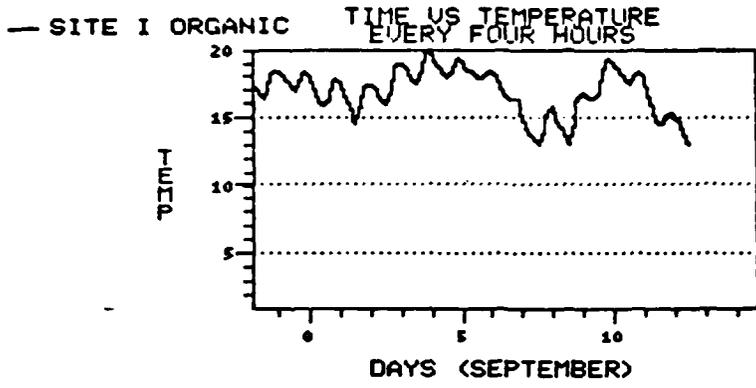


FIGURE 2. Ambient monitoring data from the control site for the organic and mineral horizons, plotted for every fourth hour.

— SITE I ORGANIC
TIME VS TEMPERATURE
ONCE A DAY

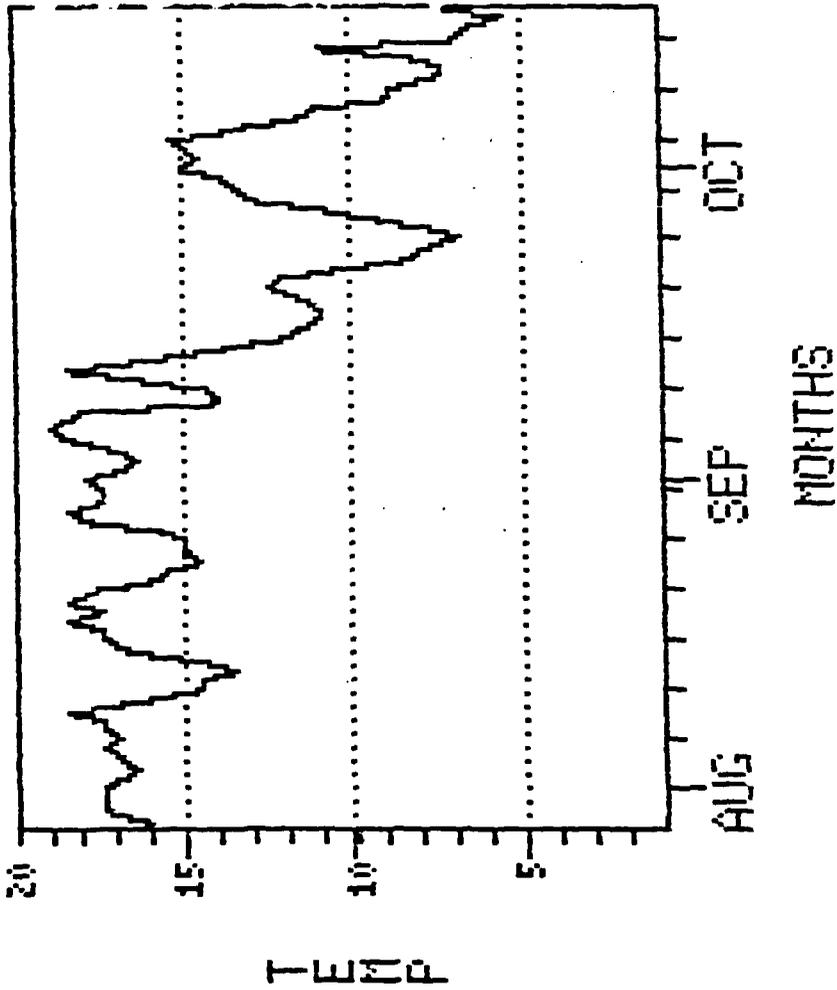


FIGURE 3a. Ambient monitoring data from the control site for the organic horizon
--temperature only, plotted once a day.

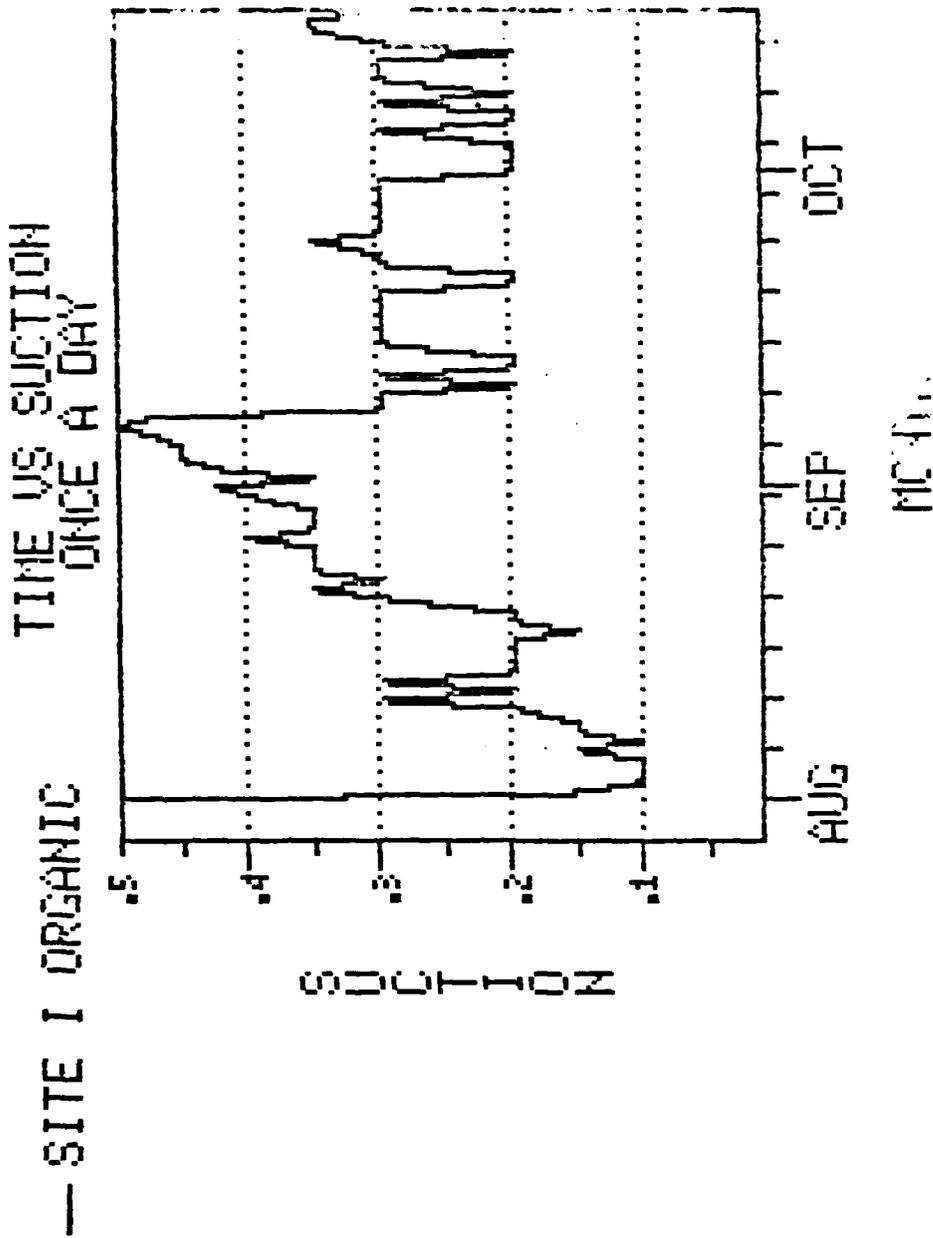


FIGURE 36. Ambient monitoring data from the control after Cap 11 breach horizon—moisture only, plotted once a day.

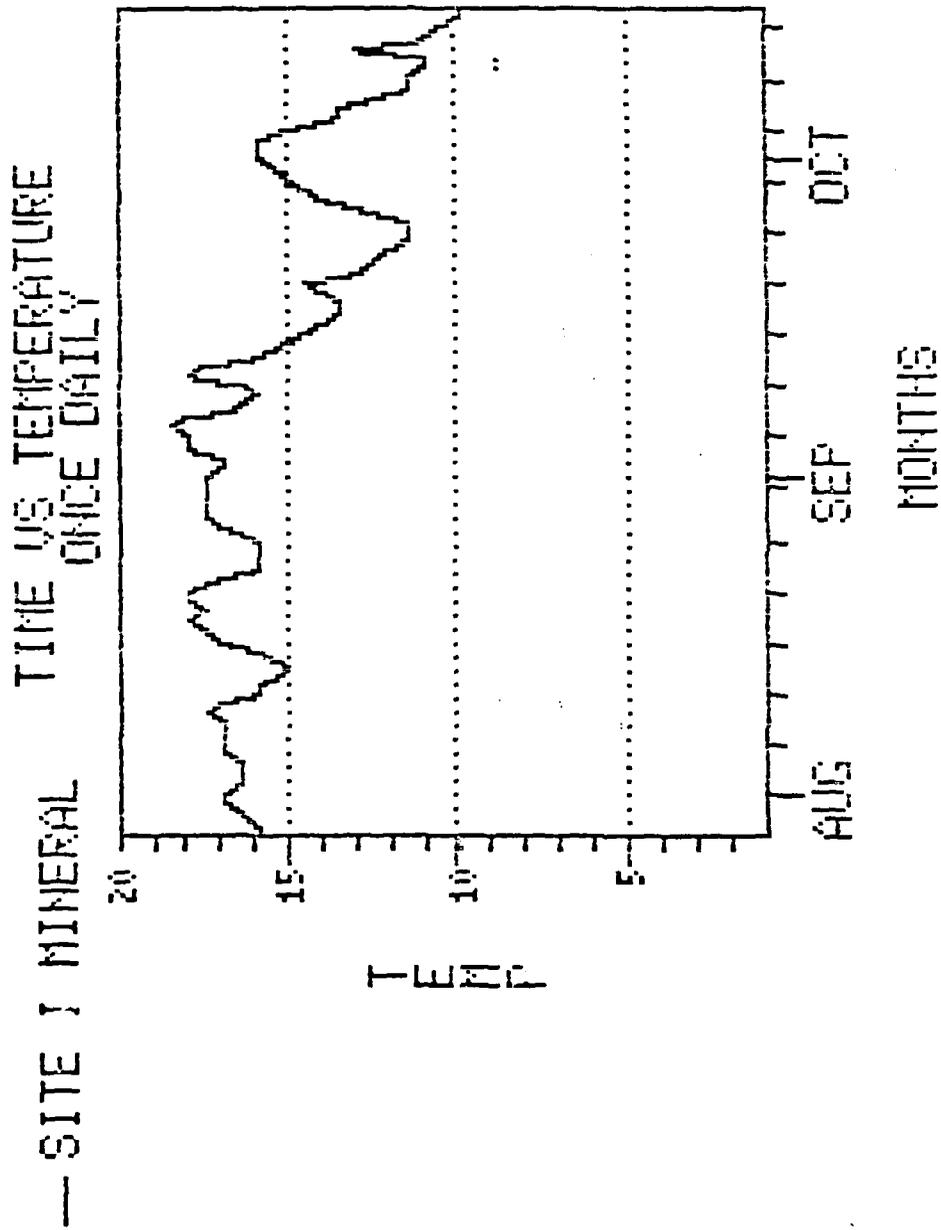


FIGURE 10. Ambient monitoring data from the control cells for the mineral rock—temperature only, plotted once a day.

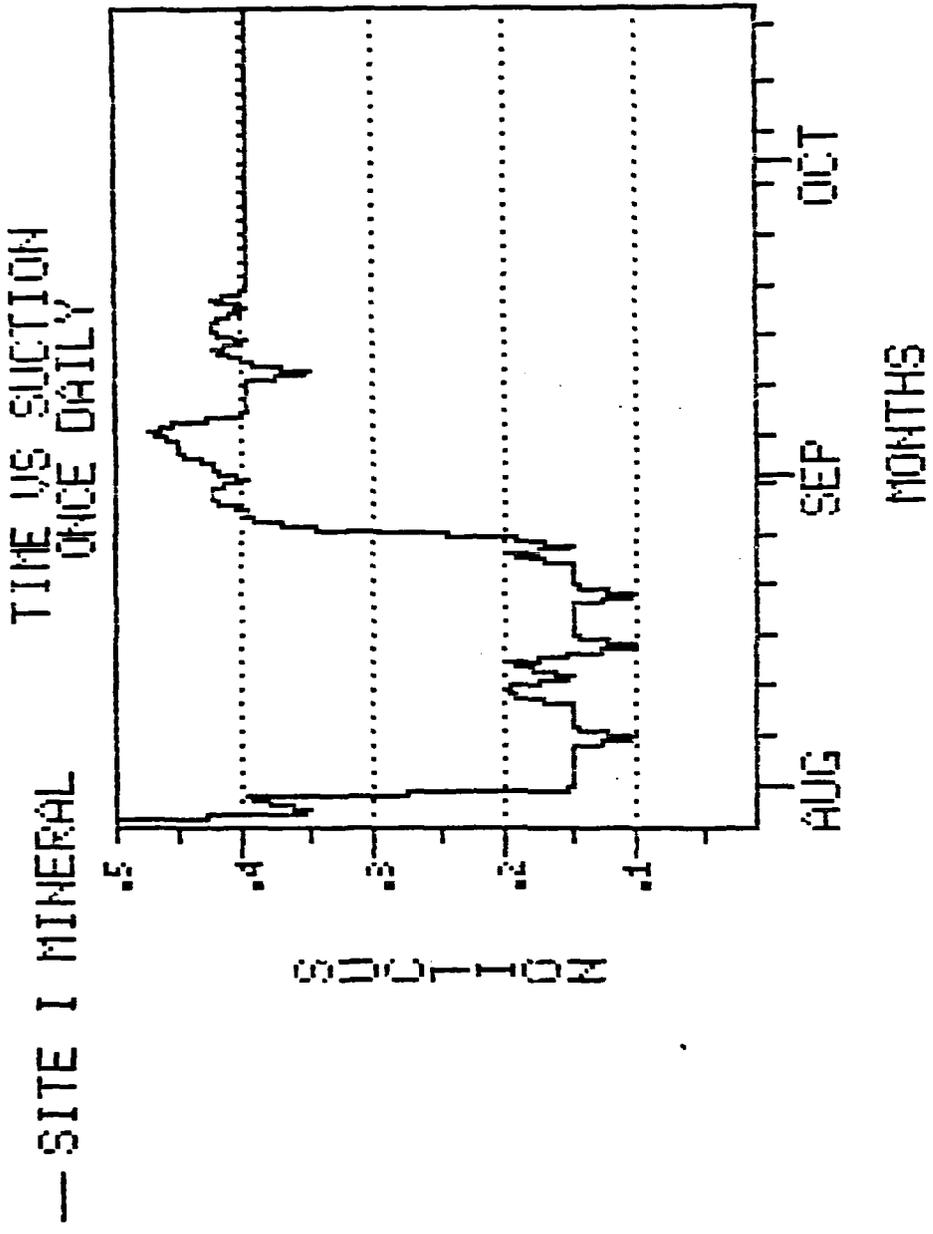


FIGURE 34. A-bient monitoring data from the control site for the mineral horizon -moisture only, plotted once a day.

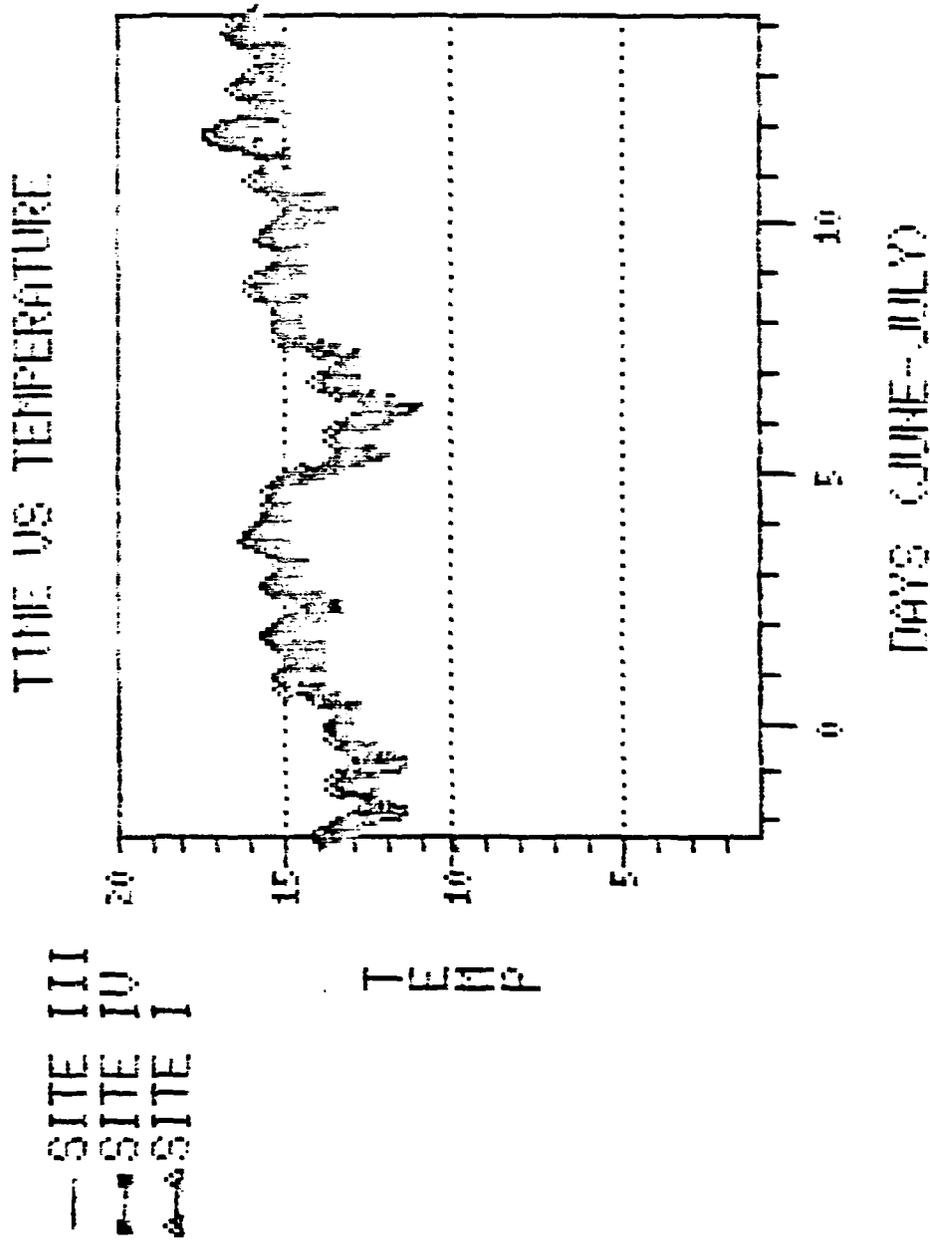


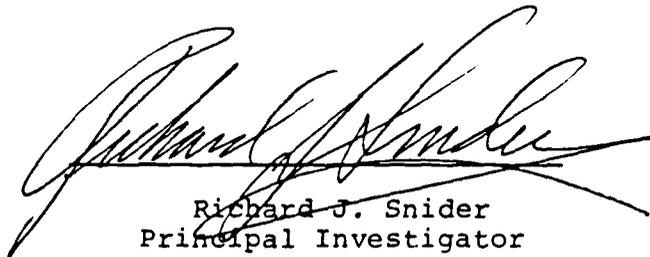
FIGURE 2. Ambient monitoring of temperature at the control (I), antenna (III) and ground (IV) sites, plotted every 4 hours.

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ELF Communications System Ecological Monitoring Program:
Soil and Litter Arthropoda and Earthworm Studies
Tasks 5.3. and 5.4.

1983



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ABSTRACT

Definitive sites (one Test, one Control), both in maple-dominated, mixed deciduous forest were selected in summer 1983. Stand characteristics (trees and shrubs) were mapped and analyzed in detail, and soils were analyzed for pH, %OM, and available macronutrients. The forest-floor invertebrate faunas were surveyed in mid-summer. All results confirmed that the two sites are ecologically well matched. Faunal sampling in a temporary site, established in 1982, was continued, and Test and Control sites were sampled from August through October. Data on arthropod and lumbricid populations, as far as available at this time, are presented. They show that all three sites harbor very similar species spectra; as a result, information obtained from the temporary site in 1982-83 will prove helpful for interpretation of faunal dynamics in definitive sites.

SUMMARY

One definitive Test and one Control site were selected in 1983, both situated in maple-basswood forest. Tree and shrub species composition and density do not differ significantly, and neither do major soil chemical parameters. Preliminary faunal surveys showed that the two sites shared many arthropod and lumbricid species, and that both sites also resembled, faunistically, the temporary sites monitored in 1982 (Turner Rd and Silver Lake).

Sampling at Turner Rd was continued in 1983. In definitive sites, faunal sampling was begun in August, after experimental design had been finalized: each site is divided into a grid of twenty 10 x 10 m quadrats, so that 20 replicate faunal samples (1/quadrat) are obtained per date. In November 1983, litter decomposition studies, using 1 mm mesh litterbags, were initiated in Test and Control.

Major accomplishments presented in this report include:

a) description of the Turner Rd soil-litter arthropod community (1982 and partial 1983 data), i.e. distribution, density and dominance relations of taxa common at, or characteristic of, the Turner Rd site; b) analysis of a 1982 pit-trapping experiment designed to quantify the technique's potential for out-trapping surface-active arthropods. c) results of an improved and validated technique for sampling lumbricid populations; d) quantitative comparison of Test/Control site characteristics (non-faunal) and preliminary species identification (lumbricids, arthropods) and description of population dynamics (lumbricids) characteristic of definitive sites. e) identification of potential sources of

variation, as well as of misinterpretation, of lumbricid population data. f) compilation of a checklist of species and families (lumbricids and arthropods) so far encountered in deciduous forests in the ELF system area.

I. INTRODUCTION

Tasks 5.3. and 5.4. are part of the multi-disciplinary Ecological Monitoring Program in Michigan's ELF system area. They deal with monitoring soil-litter arthropod and earthworm population dynamics and, to a limited extent, monitor litter breakdown processes mediated by the decomposer complex of the forest floor.

In mid-summer of 1982, we began faunal sampling in preliminary sites ("Turner Rd" as main site, "Silver Lk." as accessory site for pit-trapping experiments), even though definitive antenna location was not yet known. These pilot sampling programs were urgent - Upper Peninsula forest floor fauna is poorly known, and we anticipated difficulties in identifying many of the taxa encountered. The 1982 material indeed provided a welcome taxonomic training experience, and is beginning to yield a comprehensive data set on arthropod/lumbricid populations of northern deciduous forests in the ELF area.

Much of the information gathered from these samples is still incomplete, since phenological description of species populations (i.e. seasonal sequence of life cycles under natural conditions) requires data that span a full season or more. In order to complete the sampling cycle begun in 1982, we continued monitoring Turner Rd. invertebrates. The site was then abandoned because of its ambiguous location relative to the antenna corridor.

At the start of the 1983 field season, selection of definitive sites became top priority, while work at Turner Rd. continued. First an Antenna Test site was located. After several thousand miles of

driving in Dickinson and parts of Marquette and Iron Counties, several potential Control sites were identified. Checks of electromagnetic background radiation, soil surveys, and brief vegetational and faunal surveys narrowed the choice to a single site in late July. Since then, Test and Control have been sampled on schedule. Care was given to physical site design (a prerequisite for maximum power of resolution of analyses of variance and covariance), as well as to the quality and efficiency of sampling techniques. At the end of the 1983 season, selected litter turnover studies were initiated, scheduled to span the subsequent 18 months.

In keeping with our objectives, we are now engaged in the sorting/identification phase of samples taken during the 1983 project period. The present report therefore contains partial data sets and analyses (e.g. 1982 Turner Rd.), as well as one-point sampling and survey data (e.g. those pertaining to comparisons between Test and Control). We herein identify the directions being taken toward implementing proposed objectives, and report as fully as possible on the progress made to date. We will not reiterate the overall statistical approach (Annual Report 1982), but will state statistical methods applied to available data.

Diagrammatic summaries of proposed objectives (Fig. 1) and of the sequence of activities in the time frame of the 1983 season (Fig. 2) are provided below. These summaries place the 1983 project period in perspective to overall goals.

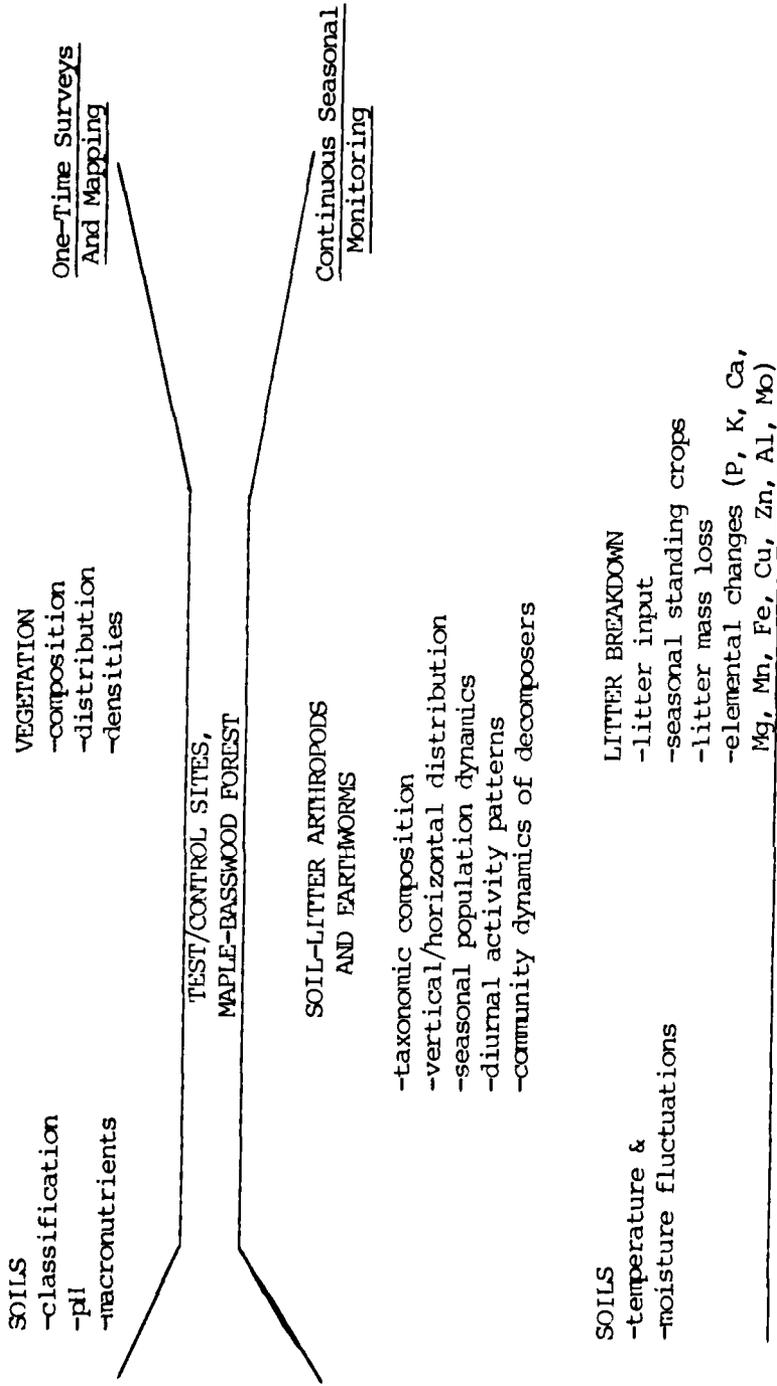


Fig. 1. Ecological monitoring objectives in Test and Control sites. Implementation of major objectives was begun in late July 1983.

1982 1983
 N D J M A M J J A S O N

LABORATORY PHASE

FIELD ACTIVITY PHASE

TURNER RD SAMPLING cont'd -----

END OF 1982 FIELD PHASE:

Faunal samples obtained at Turner Rd and Silver Lk. sites:

- Rough sorting (to order)
- Taxonomic breakdown (family, genus)
- Species identification (selected taxa)
- Optimize data management programs
- begin computerized data base, interactive with data management optimization

SITE SELECTION

TEST / CONTROL SITES:

- Site comparison: soil type and chemistry, vegetation, selected faunal parameters
- Quantitative faunal sampling
- Optimization of techniques
- Microclimatic monitoring systems installed
- Site-specific mapping programs (trees & shrubs) near completion
- Process-oriented studies initiated (litter input and breakdown)

Fig. 2. Summary of activity sequence, 1983 Project Period.

II. DEFINITIVE STUDY SITES

A. The selection process

The number of driveable roads and trails that meet the antenna corridor determine, and limit, the number and kinds of Test sites available. We therefore focused first on finding an acceptable Test area. Acceptability was measured by: presence of hardwood forest of sufficient size and homogeneity; presence of a reasonably diverse (arthropods) and dense (lumbricids) fauna; and short walking distance from the road, since we routinely take large numbers of heavy as well as easily-spilled samples.

Both DNR and Soil Conservation Service officials were extremely helpful, the former by suggesting potential study areas, the latter by on-site surveys to assess soil type homogeneity within a potential site. In June, we decided on a Test site which met our criteria and in which no lumber sales had been finalized.

We then surveyed State land, and later privately owned lands, in search of Control sites. While we could not expect to match the Test site in all respects, the major difficulty was the fact that the corridor runs through soil types which are encountered only sporadically in other parts of Dickinson and adjacent counties (i.e. at a great enough distance from the corridor to be useable as Control). We finally assessed potential sites with respect to stand age, cutting history, tree species, and A horizon development irrespective of soil type. Brief qualitative sampling of lumbricids and macroarthropods completed acceptance/rejection criteria.

Of several sites thus chosen, one was acceptable with respect to electromagnetic background radiation (IIT Research Institute, written confirmation October 1983). Detailed site layout, and quantitative faunal and vegetational monitoring, were begun in late July. Beginning with an account of site and sampling design, the season's accomplishments are presented in the following sections.

B. Site configuration and sampling design

1) The grid system:

The most uniform portion of each stand was selected in each site, large enough to accommodate at least 20 to 30 quadrats (10x10 m each), separated by 1.5 m walkways. A precise grid was then measured off and marked, resulting in well over 30 individual quadrats per site.

Based on visual assessment of homogeneity, 26 quadrats/site were chosen for long-term monitoring: 20 quadrats for sampling per se, and 6 quadrats as "check" plots (yearly photographic records of the latter will be maintained in order to document the normal development of absolutely untouched areas within the sites). Remaining squares, often interspersed between monitored ones, were rejected due to physiographic (boulders) or vegetational (obstructive and unusual amounts of downed trees, or low tree density) unconformities.

In Fig. 3, definitive grid configurations are diagrammed for Test and Control sites.

2) Sampling and monitoring design:

In order to maximize statistical power (i.e. eliminate a within-quadrat error term in ANOVA), one sample of a given type is taken from each quadrat/site/date.

Given that a 10x10 m square can be divided into 100 (1 m²) units by coordinates spaced 1 m apart, the distribution of various sampling activities (Fig. 4) was finalized as follows:

- a) Soil-litter arthropods and lumbricids:

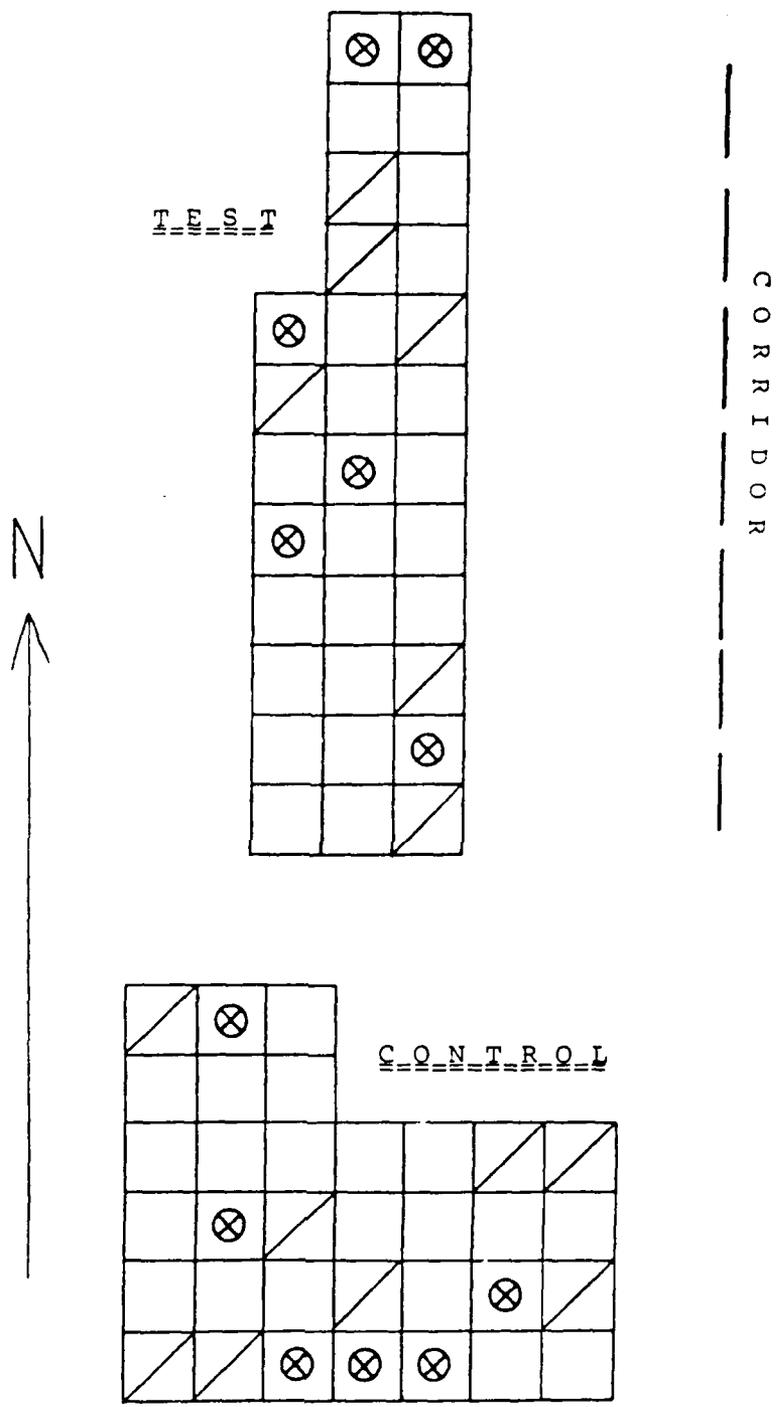


Fig. 3. Test and Control site configurations. Rejected quadrats are marked by a diagonal line; photographic checkplots are marked by an X.

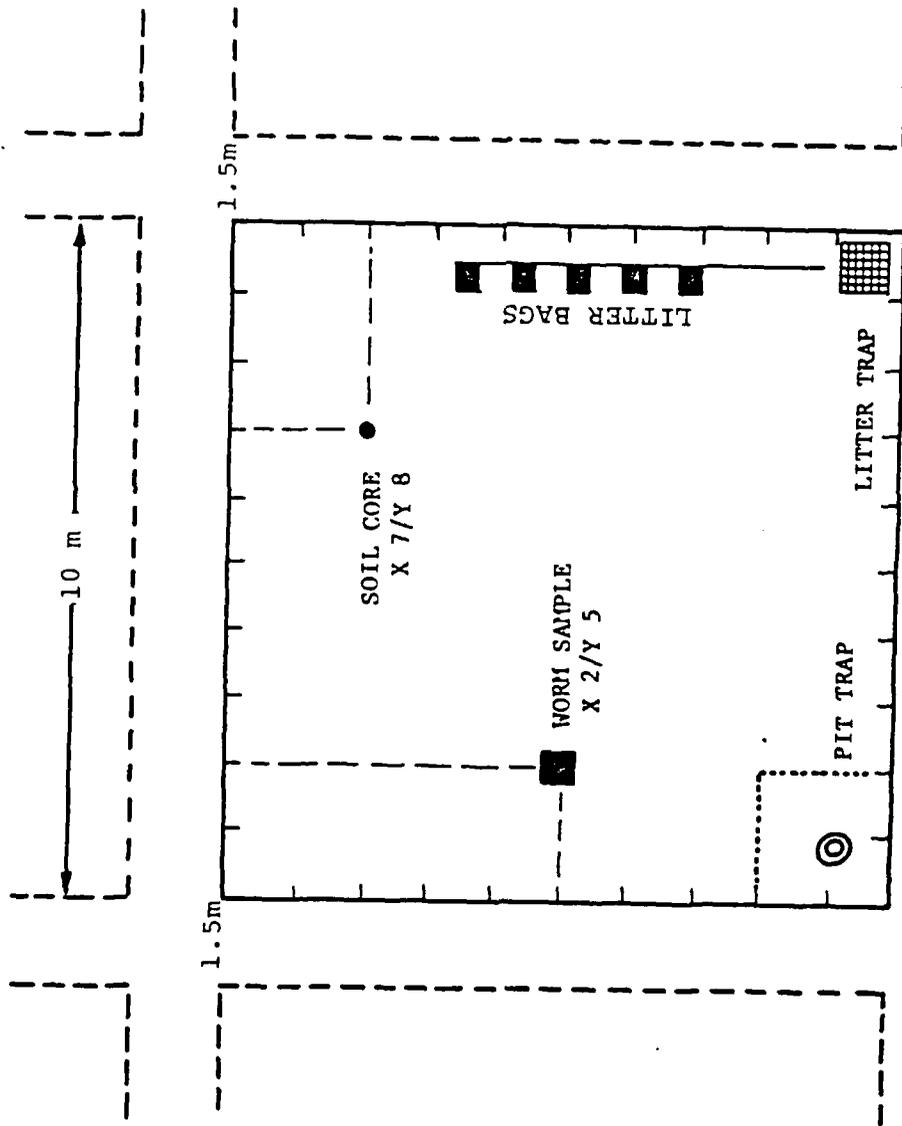


Fig. 4. Representative quadrat of the Test and Control grid systems, indicating example locations of each type of sampling activity.

Permanent pit traps are located in one corner of each quadrat such that they can be retrieved or activated without entering the quadrat itself. A total surrounding area of 4 m² (dotted line in Fig. 4) is set aside for non-use.

Exact locations of soil core, litter and earthworm samples are determined separately for each sampling date. Excluding areas reserved for permanent sampling devices, a computer-generated random number program yields one random pair of coordinates for each date and site. Twenty samples of each type, in replicate locations within each quadrat, are then taken.

b) Litter turnover:

Litter traps, 50x50 cm, are permanently placed in one corner of the quadrats, immediately adjacent to walkways (Fig. 4). Litter bags (1.0 mm mesh size) were placed in the field (location shown in Fig. 4) at the end of leaf fall. We estimated 13 sampling dates over the subsequent 18 months, with 8 bags retrieved per date. An extra 15 bags were put out to accommodate loss or destruction.

Preliminary tests had shown the existence of a potential source of variation in litter breakdown data: immatures of small litter-dwelling lumbricids were found able to enter 1 mm mesh bags. Given that: a) we wish to assess the relative contribution of arthropods vs lumbricids + arthropods to litter mass loss; but b) heat-extraction of litter bags yields arthropods but not lumbricids, then: c) colonization of decomposing litter by lumbricids represents an unknown, potentially important source of error. A validation protocol was therefore devised as follows:

Normal litter bags
(8/date, 120 in each
site since Nov. 1983)

↓
weigh

↓
heat-extract
arthropods

↓
measure litter
mass loss

↓
process litter for
elemental analysis

"Worm sham"
litter bags (9 in each
site since Nov. 1983)

↓
weigh & remove litter

↓
formalin-extract
lumbricids

↓
re-fill bags with
known quantity of
litter aged in the
field since Nov 1983

↓
replace bags in the
field for subsequent
samplings

↙ ↘
Results:
estimate of lumbricid
colonization of litter-
bag litter at x months of age

c) Microclimatic monitoring:

A set of remote sensors and recording devices were installed in each site in a centrally located quadrat. Sensors (soil temperature, soil suction) were buried at depths of 5, 10 and 20 cm. Their output is recorded continuously in storage modules which are periodically read onto computer diskettes. These devices were installed in August 1983, but several malfunctions occurred thereafter. We spent the rest of the season calibrating each temperature sensor, and identifying sources of error. We expect all systems to be functional and trustworthy in 1984.

C. Site description and comparison

1. Soils:

Soils in both sites are classified as Emmett sandy loams, slightly coarser in Test than in Control. Since the pertinent literature gives no evidence of arthropod variation due to different soils within a given type, site comparison centered on analysis of pH, OM, and available macronutrients. Results are summarized in Table 1.

Table 1. Results of soil tests, Test and Control site, for pH, %OM, and available macronutrients (kg/ha), from 10 samples of the humus (A) horizon and 10 samples at approximately 10 cm below A.

Means \pm SE (n=10)

Horizon	pH	% OM	P	K	Ca	Mg
A Control	5.7 \pm 0.1	9.3 \pm 0.7	46.1 \pm 3.7	162 \pm 13	3501 \pm 341	272 \pm 23
A Test	5.9 \pm 0.2	9.6 \pm 1.6	65.0 \pm 8.5	179 \pm 24	3293 \pm 483	237 \pm 39
	NS	b) NS	a)P <0.1	b) NS	NS	b) NS
B Control	5.7 \pm 0.1	2.0 \pm 0.2	72.9 \pm 11.1	76 \pm 10	1176 \pm 171	163 \pm 16
B Test	5.9 \pm 0.2	2.7 \pm 0.2	51.2 \pm 11.3	79 \pm 3	1312 \pm 241	98 \pm 20
	NS	NS	NS	NS	NS	NS

NS = not significant (P>0.2)

a) Lohrding's (1969) test (variances unequal, coefficients of variation equal)

b) Behrens' (1929) t-test, using Welch's (1938) approximate degrees of freedom (variances and coefficients of variation unequal)

All others tested by two-sample t-test.

With the possible exception of available P (to be further measured in the 1984 season), none of the major soil chemical parameters differed significantly in Test and Control sites. These results further substantiated our choice of this particular Control to be paired with the Test site: in many other areas of Dickinson Co., especially the southern and eastern parts, Ca levels are known to be much higher than in soils in the immediate vicinity of the antenna corridor (Walter P. Summers, Soil Conservation Service, pers. comm.).

2. Vegetation

Description of the hardwood stands in Test and Control provides meaningful ecological comparison between sites. In 1983, surveys of tree and shrub populations were performed in up to 80% of the grid quadrats in each site. These surveys, (to be completed in 1984 with inclusion of ground cover composition and distribution), represent the beginning of a detailed vegetation mapping program.

For descriptive purposes, the three parameters of major importance are density, frequency, and cover or basal area (Mueller-Dombois and Ellenberg 1974). In sixteen 10x10 m quadrats (sample units), trees (≥ 12.7 cm dbh), understory (1.0 - 12.6 cm dbh) and shrubs were mapped according to x/y coordinates. For clarity, we first present a species list (Table 2) indicating presence/absence in Test and Control sites; common names will be used thereafter.

Table 2. Species list for trees and shrubs, and presence of each, in Test and Control sites.

Species		Site presence
Sugar maple	<u>Acer saccharum</u> Marsh	T,C
Basswood	<u>Tilia americana</u> L.	T,C
Elm	<u>Ulmus americana</u> L.	C
Hornbeam	<u>Ostrya virginiana</u> (Mill). K. Koch	T,C
Quaking aspen	<u>Populus tremuloides</u> Michx.	T
Yellow birch	<u>Betula lutea</u> Michx.f.	C
Bigtooth aspen	<u>Populus grandidentata</u> Michx.	C
Leatherwood	<u>Dirca palustris</u> L.	T,C
Hazelwood	<u>Hamamelis virginiana</u> L.	T,C
Willow	<u>Salix</u> sp.	C
Gooseberry	<u>Ribes</u> sp.	T
Serviceberry	<u>Amelanchier canadensis</u> (L.) Medic.	T
Balsam fir	<u>Abies balsamea</u> (L.) Mill.	T,C

Quantitative comparisons are possible in several ways; Table 3 lists two approaches: actual counts/total sample area of 1600 m², which are very similar in the two sites (means of 16.2±2.3, and 13.1±1.8, for understory, were not significantly different); and average basal area/tree, tested with appropriate t-statistics after testing homogeneity of variances.

Table 3. Tree and understory characteristics in Test and Control: total counts/1600 m² (16 quadrats) and mean basal areas $\bar{x} \pm SE$ for the common species.

	Actual no./1600 m ²		Basal area $\bar{x} \pm SE$		
	Test	Control	Test	Control	Sign.
Maple trees	73	72	241.4 \pm 12.3	243.5 \pm 13.8	NS
underst.	259	208	35.2 \pm 1.8	40.1 \pm 2.4	NS
Basswood trees	33	26	524.2 \pm 47.5	416.7 \pm 40.0	P<0.1
underst.	4	14	92.4 \pm 10.0	58.4 \pm 6.6	
Hornbeam trees	1	-	-	-	
underst.	4	5	111.8 \pm 18.5	91.4 \pm 15.0	NS
Elm trees	-	6	-	219.1 \pm 52.6	
underst.	-	23	-	49.7 \pm 7.3	
Quaking aspen	4	-	-	439.2 \pm 85.0	
Bigtooth aspen	-	2	-	-	
Yellow birch	-	1	-	-	
Poplar	-	1	-	-	

Maple basal areas did not differ, but basswood trees in Control were somewhat at less mature and less dense than in Test. Hornbeam is a minor species in both sites, while elm, entirely absent in Test, is represented mainly by understory individuals in Control. It is indeed the presence of American elm in Control that has the greatest effect on different importance values (Greig-Smith 1964) for each community (Table 4).

Table 4. Importance values (= relative density + relative dominance + relative frequency) of tree species in Test and Control communities.

<u>Species</u>	<u>Test</u>	<u>Control</u>
Maple	195.1	166.6
Basswood	82.0	64.1
Elm	-	37.5
Hornbeam	11.7	6.9
Bigtooth aspen	-	6.9
Yellow birch	-	4.2
Poplar	-	3.4
Quaking aspen	11.2	-

Shrub and seedling (<1 cm dbh, \geq 50 cm high) distribution was recorded along x/y coordinates for 16 quadrats in Test, and 13 quadrats in Control. Densities and frequencies are shown in Table 5. Only three species are common in that stratum with leatherwood dominant, in both sites. All others, including small seedlings of maple, balsam fir and aspen, were represented by single or few individuals/total area surveyed.

Table 5. Shrub and seedling populations in Test and Control; (frequency, in parentheses, = no. of quadrats in which a species occurs/total quadrats sampled). Leatherwood densities: not significantly different ($P < 0.2$).

$$\bar{x}/100\text{m}^2 \pm \text{SE}$$

Species	Test	Control
Leatherwood	5.25 \pm 1.73(0.75)	8.23 \pm 1.87(0.92)
Hornbeam	2.13 \pm 0.75(0.69)	2.46 \pm 0.60(0.77)
Hazelwood	0.75 (0.13)	0.46(0.23)
Willow	-	3.15 (2 clumps)
Serviceberry	1/1600 m ²	-
Gooseberry	1/1600 m ²	-
Balsam fir	1/1600 m ²	2/1300 m ²
Maple	-	4/1300 m ²
Bigtooth aspen	-	1/1300 m ²

Our last parameter, potentially differing between sites, was mapped in the same manner as trees and shrubs: the number and distribution of standing dead. In Table 6, total numbers/1600 m², and average dbh of standing dead are given for Test and Control

Table 6. Number and average size of standing dead in Test and Control (observed totals from 1600 m²).

Species	cm dbh $\bar{x} \pm \text{SE}, (n)$	
	Test	Control
Maple	4.5 \pm 0.2 (87)	5.1 \pm 0.4 (50)
Basswood	- (1)	8.6 \pm 0.6 (10)
Elm	-	5.6 \pm 0.5 (17)
Total others	(7)	(1)

In Control, both elm and basswood contribute to standing dead; Test contains virtually dead maple alone, in greater numbers but of smaller size than in Control. However, neither mean dbh ($P < 0.1$), nor number of dead maples/quadrat ($P < 0.1$), nor standing dead of all species/quadrat ($P < 0.15$) can be said to differ to any significant degree.

3. Litterfall

An initial comparison of litter inputs was possible in 1983 once litter traps had been installed in Test and Control (Fig. 5). Maple leaf fall was more strongly bimodal in Control, and "other" species exhibited a more pronounced peak late in the season. Cumulated over all dates and species, total dry wt. litter inputs were 310 g/m^2 in Control and 283 g/m^2 in Test. In keeping with density and basal area measurements reported earlier, basswood litter input was higher, although not significant by so, in the Test site (Fig. 5).

4. Faunal parameters

Below we present a preliminary checklist of families and species found in either or both Test and Control sites (Table 7). It consists of taxa which could be rapidly collected and identified, many of them known to be numerically the most abundant in the species assemblage (e.g. Collembola, Carabidae, Chilopoda, Lumbricidae). No absolute densities are given in Table 7: quantitative faunal parameters will not become available until 1983 samples have been completely sorted and identified.

Provenance of the faunal data used in Table 7 is as follows:

- Lumbricidae: selected quantitative, handsorted samples (see also Section F);

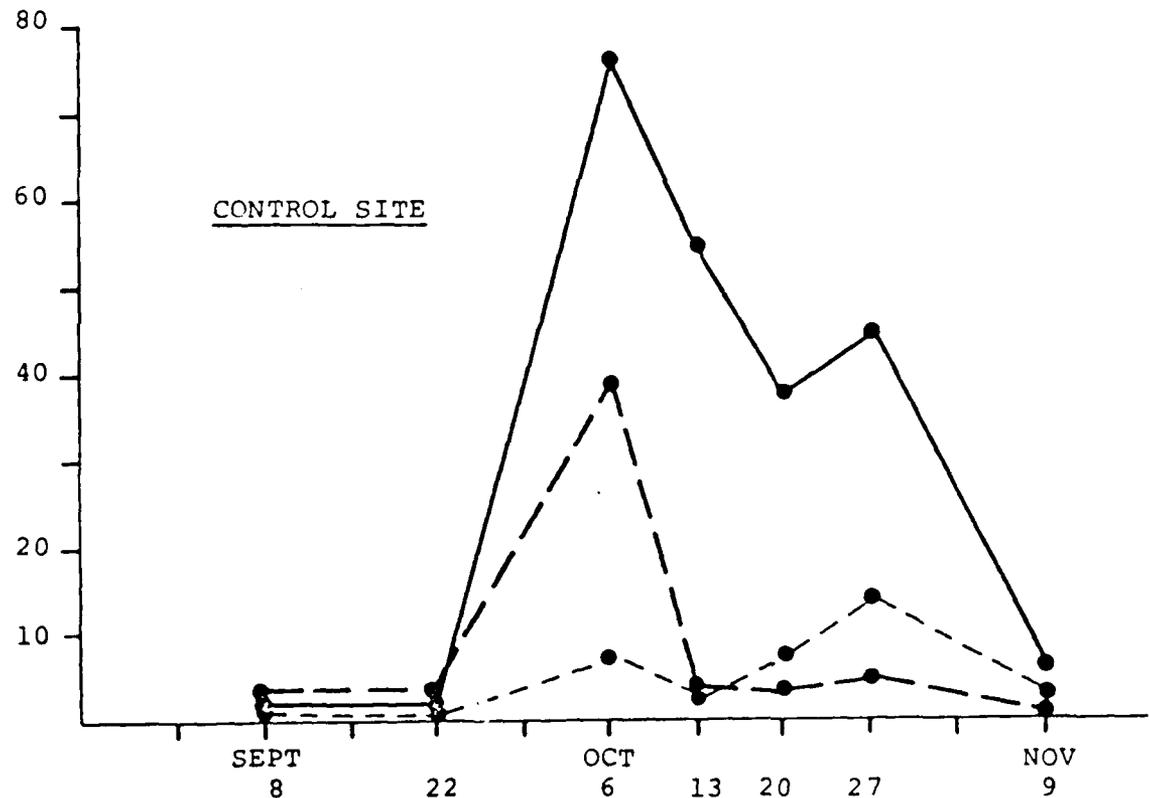
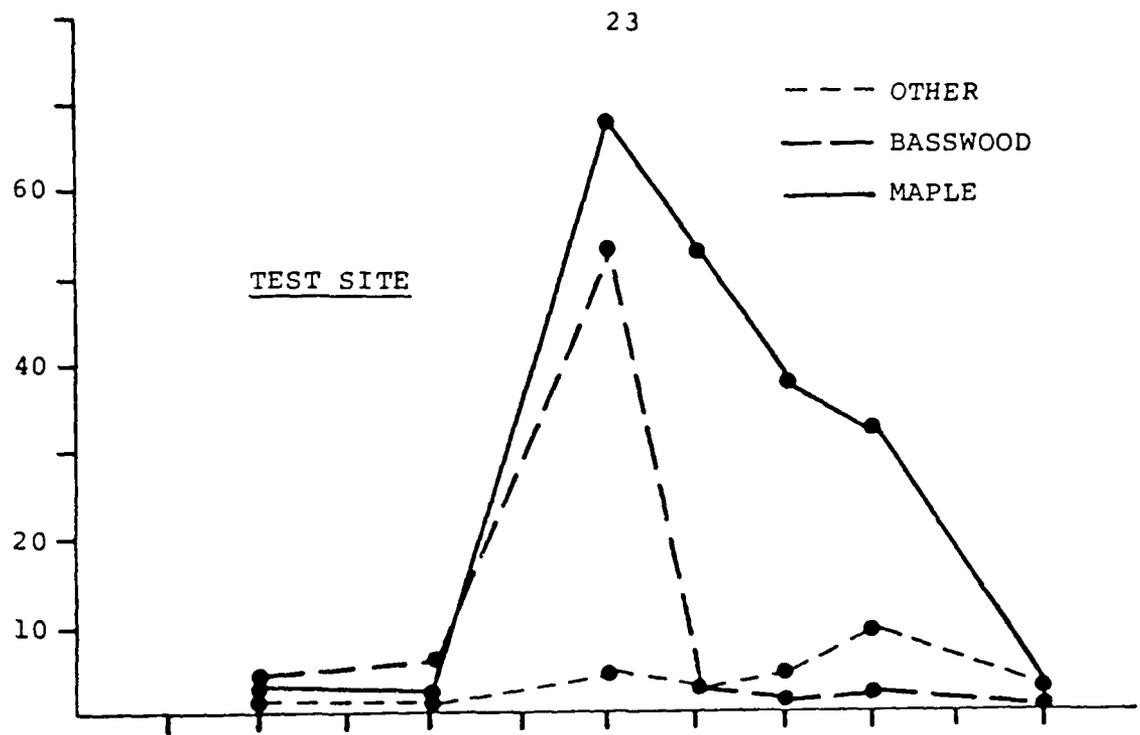


Fig. 5. Litterfall, in Test and Control, 1983: average g/m², obtained from 20 litter traps, on the specific collection dates shown on the x axis.

- Myriapoda: hand-collecting on site, and lumbricid samples;
- Coleoptera, Diptera, Collembola, Orthoptera, Opiliones: two consecutive days of pit-trapping at the time of final site selection (late July).

The checklist is necessarily incomplete, and frequency ratings may be biased by a taxon's seasonality of occurrence; however, these data provide an initial gross comparison between Test and Control faunas. Many taxa are common to both sites, and "probable dominants" (+++) within a given group are often represented by the same species.

Table 7. Tentative checklist of lumbricid and arthropod taxa known to occur in litter and soil in Test and Control sites. Frequency ratings (+++ to -) are relative to other members of the same family or order, and are based only on late-summer sampling and collecting.

Taxon/species	Tentative frequency rating	
	Test	Control
<u>Lumbricidae:</u>	++	+++
<u>Dendrobaena octaedra</u> (Savigny)		
<u>Lumbrius rubellus</u> (Hoffmeister)	+++	++
<u>Aporrectodea longa</u> (Ude)	+	+
<u>A. tuberculata</u> (Eisen)	+++	-
<u>A. turgida</u> (Eisen)	-	+++
<u>Opiliones:</u>		
<u>Leiobunum nigripes</u> (Weed)	++	+
<u>L. nigropalpi</u> (Wood)	+	-
<u>Sabacon crassipalpi</u> (Koch)	+	-
<u>Odiellus pictus</u> (Wood)	+	-
<u>Myriapoda:</u>		
<u>Diplopoda:</u>		
<u>Uroblaniulus canadensis</u> (Newport)	++	++
<u>Cleidogona exaspera</u> (Williams & Hefner)	++	++
<u>Polyzonium bivirgatum</u> (Wood)	+	+
<u>Chilopoda:</u>		
<u>Taiyuna opita</u> (Chamberlin)	+++	++
<u>Strigamia chionophila</u> (Wood)	++	+++
<u>S. branneri</u> (Bolles)	+	+
<u>Geophilus</u> sp.	+	-
<u>Nadabius iowensis</u> (Meinert)	+	+
<u>Lithobius forficatus</u> L.	+	-
<u>Collembola:</u>		
<u>Entomobryidae</u>		
<u>Tomocerus flavescens</u> (Tullberg)	+++	+++
<u>T. lamelliferus</u> (Mills)	+	-
<u>Orchesella hexfasciata</u> (Harvey)	+++	++
<u>O. ainsliei</u> Folsom	+	-
<u>Lepidocyrtus paradoxus</u> (Uzel)	-	+
<u>Pseudosinella violenta</u> (Folsom)	++	++
<u>Entomobrya comparata</u> (Folsom)	++	++
<u>E. nivalis</u> (Linnaeus)	+	+
<u>Sminthuridae:</u>		
<u>Sminthurinus henshawi</u> (Folsom)	+++	+++
<u>Sminthurides lepus</u> (Mills)	-	+
<u>Dicyrtoma mamorata</u> (Packard)	+	+
<u>Arrhopalites benitus</u> (Folsom)	+	-
<u>A. amarus</u> (Christiansen)	+	-
<u>Isotomidae:</u>		
<u>Isotoma viridis</u> (Bourlet)	+	-
<u>I. nigrifrons</u> (Folsom)	++	++

Table 7. cont'd

Taxon/species	Tentative frequency rating	
	Test	Control
Coleoptera:		
Carabidae		
<u>Pterostichus melanarius</u> (Illiger)	+++	+++
<u>P. coracinus</u> (Newman)	++	+++
<u>P. pennsylvanicus</u> (Leconte)	+	++
<u>Synuchus impunctatus</u> (Say)	+	++
<u>Calathus ingratus</u> (Lejean)	-	++
<u>Calathus gregarius</u> (Say)	+	-
<u>Clivina fossor</u> (Linne')	+	-
Scarabaeidae		
<u>Geotrupes</u> sp.	+	+
Staphylinidae		
	+++	+++
Leiodidae		
	++	++
Eucnemidae		
	+	+
Cryptophagidae		
	+	-
Nitidulidae		
	-	+
Colydiidae		
	-	+
Curculionidae		
	-	+
Orthoptera:		
<u>Ceuthophilus brevipes</u> (Scudder)	++	++
Diptera:		
Phoridae (Wingless)	+	++

III. DEFINITIVE AND TEMPORARY SITES: RESULTS AND PROGRESS

A. Introduction

In earlier sections we have given analyses of non-faunal parameters obtained for Test and Control sites, as well as an account of how litter decomposition objectives were implemented. In the following sections, we present selected sets of faunal data derived mainly from 1982 work, since 1983 samples from all sites are not completely sorted at this time. Available numerical results and analyses fall into three major categories:

a) "Silver Lake" pit-trapping data: a study conducted August to October 1982, at a hardwood site abandoned thereafter. Its purpose was quantification of the effect of linear distance between traps on numerical depletion of surface - active species.

b) "Turner Rd." faunal data for 1982 and, in selected cases, 1983: population dynamics of arthropods and analysis of seasonal patterns of surface - activity. As discussed earlier, continued sampling at Turner Rd. (1983) will allow us to quantify biological and ecological parameters for many species which occur in all sites. Given that only late - season 1983 samples are available from Test and Control, Turner Rd. data offer the obvious advantage of giving us "advance" knowledge on these shared species and their early - season dynamics. Where appropriate, faunal similarities between Test, Control, and Turner Rd. will be pointed out.

c) Current knowledge on lumbricid species assemblages in the ELF system area, using Turner Rd. data as background, and emphasizing

ecological analysis of the species populations in Test and Control sites.

Finally, we have compiled a list of new geographic distribution records for species, and a checklist of families, genera, and species, so far encountered in the ELF system area (Appendices A and B). In the presentation of results in subsequent sections, generic designations will usually be abbreviated. For full species names, refer to the preliminary formal checklist given in Appendix A.

B. Summary of sampling activities

In Table 8, 1983 sampling schedules are summarized for Turner Rd. as well as for definitive sites. Even for labor - intensive types of sampling activities, we were able to adhere to a high - frequency schedule. Starting dates as shown in Table 8 reflect the period of Test/Control site selection (May - July), resulting in faunal samples being taken only in the latter part of the summer.

C. Technique validation: pit-trapping at Silver Lake, 1982

1. Introduction:

Pit-trapping is probably the most widely used method for monitoring surface-active arthropods. Catches are often difficult to interpret, since they are a compounded result of population size and activity (Mitchell 1963; Greenslade 1964; Ericson 1979), and vary with physiological state of the animals (Ericson 1977, 1978).

Trap catches are also greatly influenced by the specific position of traps relative to each other. In a grid system, usually with distances of 5 m between traps (Ericson 1979; Luff 1975), inner traps are masked by outer ones and catch fewer animals. Trapping

Table 8. 1983 Summary totals of Arthropod and Lumbricid sampling programs in Turner Rd., Test, and Control sites. Starting dates differed between temporary (Turner) and definitive sites, while sampling frequencies and replications were the same in all sites.

Sample type	Starting Date		Frequ., days	Rep./ date	N Dates		N Samples
	Turner	Test/Con			Turner	T/C	
Lumbricid 1/16 m ² sort/sieve	May 8	Aug. 8	14-21	20 (x 4 subs.)	11	5	320
soil cores, heat extr.	May 4	July 28	14	20	12	6	360
Litter 1/16 m ² heat extr.	May 14	July 28	14	20	12	6	360
Pit traps, diel	May 4	July 27	7	20 (x 2 subs.)	25	13	1520

along transects, less likely to result in over-exploitation, may produce larger initial catches (Barlow 1957). The possibility of over-trapping, and the magnitude of its effects, is likely to vary with distance between traps (Fairhurst 1979; Cotton and Miller 1974), superimposed on initial density and behavioral characteristics of the trapped animals.

Knowing that pit-trapping would be one of our major research tools, we decided to quantify the inherent potential of the method for out-trapping arthropods. As soon as a preliminary survey of forests in the ELF system area was underway in 1982, we therefore selected a site for that purpose. The site ("Silver Lake") was representative of other candidate sites in which definitive project objectives could be implemented.

The design of this experiment had two essential features: a) trapping was done along transects because the site consisted of an elongate ridge; i.e., transect - trapping would have been chosen in any similar site used for long-term studies. And b) the trapping schedule was relatively intensive, in terms of intervals between samplings and of distances between traps.

In the following sections, we present results of the Silver Lake study. Their interpretation is enhanced, at least for certain arthropod taxa, by data obtained simultaneously in the second temporary study site (Turner Rd.). Both sites, although botanically somewhat dissimilar, share a number of arthropod species with comparable species dominance characteristics.

2. Methods:

Conforming to the relief of the site, four trapping transects were established along its ridge. Each transect consisted of a linear series of 12 traps such that the distance between any two transects was at least 10 m. All transects faced only one neighboring one, and thus were "open" on the other side.

Distances between traps were as follows:

Transect 1: 4.0 m

Transect 2: 2.0 m

Transect 3: 1.0 m

Transect 4: 0.5 m

All traps were activated and emptied during 5 consecutive 24 hour periods, at intervals of 3 weeks; i.e., 5 days of trapping August 3-7, August 26-30, September 13-17, and October 4-8. Twenty samples were thus obtained from each individual trap over a nine-week period.

All traps were installed one week prior to the first trapping date, to eliminate the "digging-in effect" (Joosse and Kapteyn, 1968) which can lead to disproportionately large initial catches. All transects were approached along the same pathway at all times, as recommended by Joosse (1965), and were handled from a distance of ≥ 0.5 m.

Ethylene glycol was used as trapping medium, in uncovered, clear plastic cups (8.5 cm diameter) whose rim was flush with the soil surface.

Arthropod catches were first sorted to order level, selected

taxa were further identified to family (e.g. Acari) or species. Results reported below do not include catches of winged Diptera, Lepidoptera and Hymenoptera, because these are not considered to be truly surface-active.

3. Results and discussion:

3.1. Total numbers trapped:

The experiment was designed to test the hypothesis that arthropod catches would decline in transect 4 to a relatively greater degree, or more rapidly, than in other transects; i.e., given that seasonal reductions in numbers could be expected to occur, that these reductions would be accentuated by increasingly severe exploitation. Using summary totals over each 5-day trapping period, it can be shown that such an effect did not materialize. Means given in Table 9 do not even require testing.

Table 9. Mean (\pm SE) number of arthropods caught per total 5-day period in each transect.

DATE	TRANSECT			
	1 (4.0 m)	2 (2.0 m)	3 (1.0 m)	4 (0.5 m)
Aug. 3-7	111.1 \pm 14.4	101.3 \pm 11.7	105.1 \pm 8.1	105.2 \pm 7.5
Aug. 26-30	43.2 \pm 4.7	37.2 \pm 12.8	40.2 \pm 5.7	28.4 \pm 4.8
Sept. 13-17	37.3 \pm 1.8	34.3 \pm 4.0	37.6 \pm 3.2	32.3 \pm 3.8
Oct. 4-8	32.0 \pm 3.9	28.9 \pm 2.4	34.3 \pm 1.8	27.7 \pm 2.0

Initial catches and relative decreases as time progressed, were essentially equal for all transects.

With respect to total arthropod catches, trap-distance effects could therefore not be proven. Neither could it be said that, within each trapping period, numbers caught decreased from day 1 through day 5 (examples of mean catches/transect/day are given in Table 10).

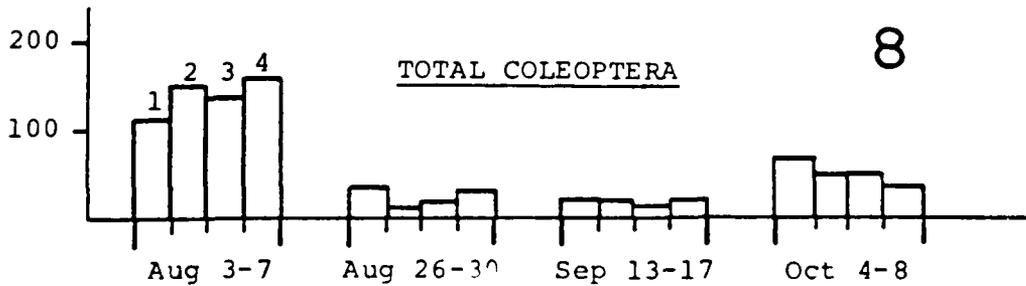
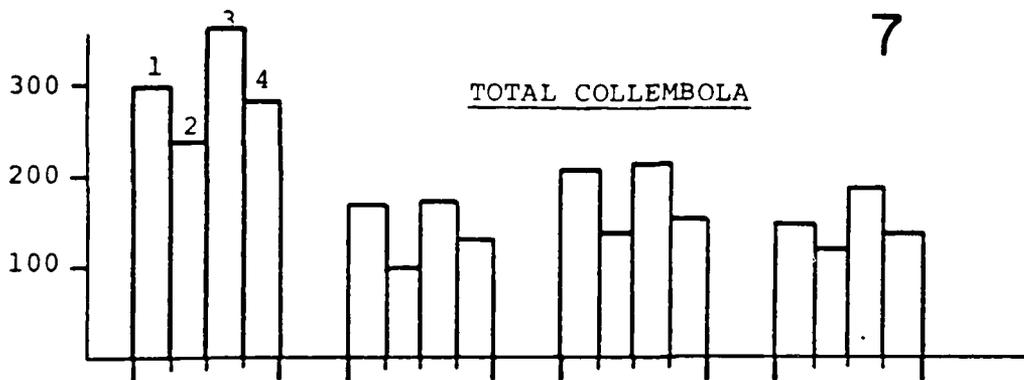
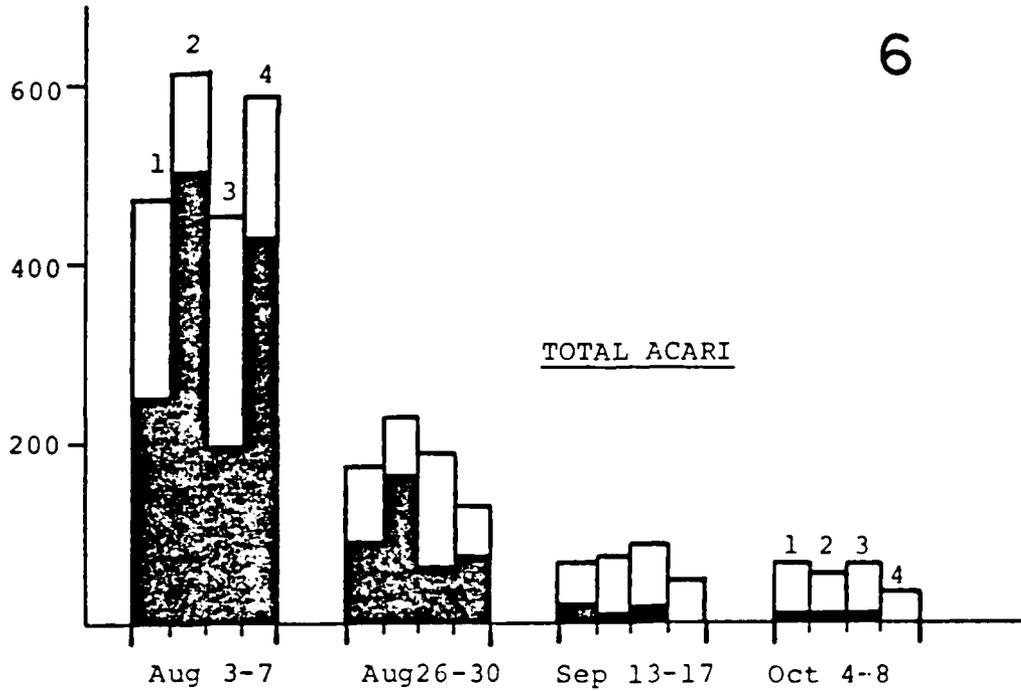
Table 10. Mean (\pm SE) arthropods caught/day and/transect (n=12 traps) during the first and last trapping period.

Period	Day	TRANSECT			
		1	2	3	4
Aug. 3-7	1	12.2 \pm 2.3	10.8 \pm 2.5	12.4 \pm 1.3	14.3 \pm 2.3
	2	31.8 \pm 9.0	32.4 \pm 8.4	35.4 \pm 6.0	23.5 \pm 5.0
	3	22.2 \pm 6.7	16.9 \pm 2.6	18.3 \pm 3.5	22.9 \pm 4.3
	4	21.5 \pm 6.7	21.2 \pm 5.6	14.1 \pm 1.7	20.1 \pm 3.4
	5	22.7 \pm 3.4	20.1 \pm 2.6	23.3 \pm 4.2	25.3 \pm 7.5
Oct. 4-8	1	6.9 \pm 1.3	7.6 \pm 1.3	8.9 \pm 1.2	8.3 \pm 1.1
	2	5.0 \pm 1.0	6.8 \pm 1.2	5.2 \pm 0.7	6.3 \pm 0.8
	3	6.6 \pm 1.4	4.5 \pm 0.6	6.4 \pm 0.8	4.3 \pm 0.5
	4	5.8 \pm 0.9	4.9 \pm 0.7	6.2 \pm 0.9	4.2 \pm 0.4
	5	6.8 \pm 1.0	5.2 \pm 0.8	7.3 \pm 1.3	4.6 \pm 0.7

3.2. Numerically abundant or constant taxa:

Taxa discussed in this section, taken all together, represent approximately 85% of each catch. The remaining 15% were excluded from analysis because of low numbers (e.g. snails, ants, chilopods, Psocoptera, Orthoptera), and/or difficulties in their identification (e.g. Diptera larvae, immature Homoptera).

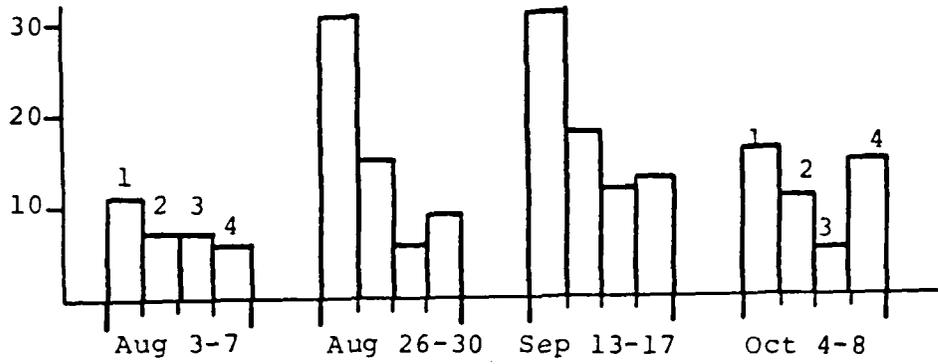
For each transect, total numbers of major taxa caught per 5-day period are shown in Figures 6-11. Overall, Collembola, mites and



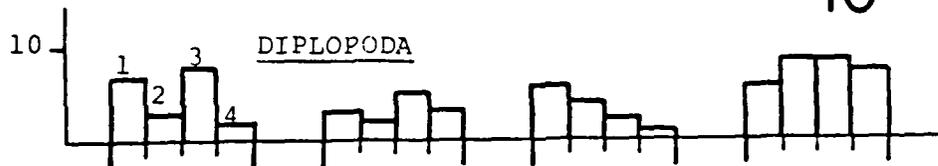
Figs. 6-8. Totals trapped in 4 trapping periods (5 days each) at Silver Lake 1982. 1-2-3-4 = transect no. Black portion of bars (Acari) = no. of hypopi of Astigmata.

OPILIONES

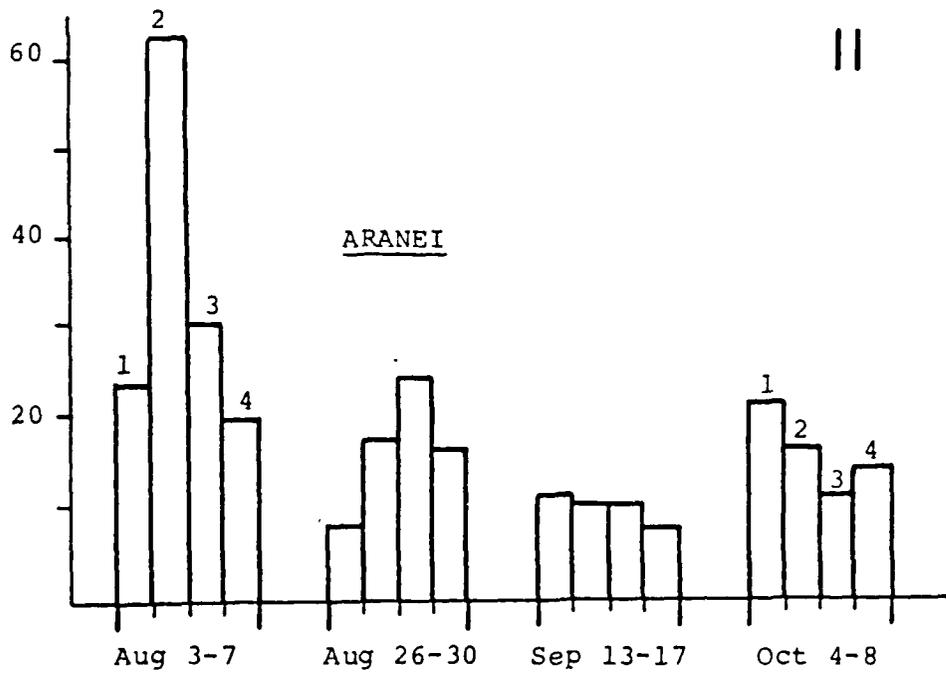
9

DIPLOPODA

10

ARANEI

11



Figs. 9-11. Total number of animals trapped in transects 1-2-3-4, summed over 5 days per trapping period, Silver Lake 1982.

Coleoptera constituted the bulk of all catches (Figs. 6,7,8). Again it becomes clear that the largest catches occurred over the first period in early August. However, subsequent changes were not always declines (e.g. Fig. 9).

Included in Fig. 6 (solid black portions) are counts of hypopi, i.e. phoretic deutonymphs of Astigmata, virtually all belonging in the family Acaridae. Hypopi do not actively enter traps, but rather release their hold on carrier hosts (other arthropods and an occasional shrew) when the latter are immersed in ethylene glycol. Numerically, hypopi often exceeded all other mites combined, and showed a pronounced seasonal decline (Fig. 6): a relationship between numbers of carriers and numbers of hypopi present in traps probably exists, but cannot be quantified meaningfully after the deutonymphs have released their hold.

Across all taxa depicted (Fig. 6-11), there appears to be no consistent effect of any one transect versus another; a possible exception is found in Collembola (Fig. 7), in which catches in transects 1 through 4 maintained a strikingly constant proportionality through all periods. This suggests that within - site location of each transect may be a factor more important than (or at least confounded with) distance between traps within transects.

3.3. Common families and species: seasonal occurrence and activity

It has been cautiously suggested (Ericson 1979) that an "approximate" knowledge of species density can be obtained by trapping. We will not draw conclusions about absolute population densities, since they would have had to be validated by area-specific

litter and soil sampling.

We can, however, use density estimates for certain species monitored at another, nearby site (Turner Rd.) - a site where A. saccharum dominates (rather than P. grandidentata), and where ground cover is less dense, but where certain arthropod species are equally as common as at Silver Lake.

Turner Rd. arthropods were monitored by extracting 1/16 m² litter samples, as well as by pit-trapping. With caution, these data (details in Section D) will be used as an explanatory or supportive base for Silver Lake results.

a. Collembola:

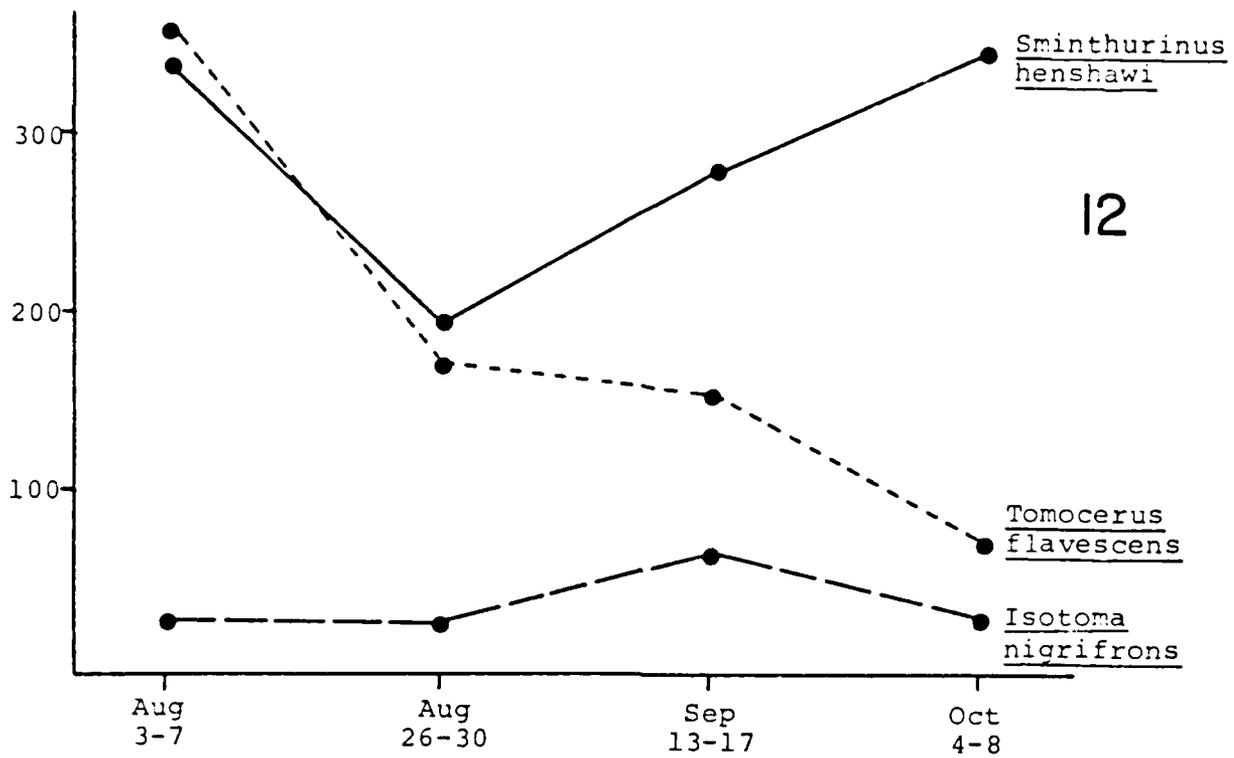
Sminthuridae, Entomobryidae and Isotomidae are families whose members frequent litter and soil surface. Catches of the dominant species of each family, summarized as totals over all transects/period, are shown in Fig. 12.

No consistent transect effect could be shown for any of the species (Fig. 13, 14, 15). S. henshawi, at Turner Rd., exhibited high densities in August and September, then abruptly (October 4) declined to 25% of September densities. Highest trap catches, however, were obtained on September 29 - lending credence to the interpretation that increased October catches at Silver Lake (Fig. 13) were due to high activity, not increased numbers.

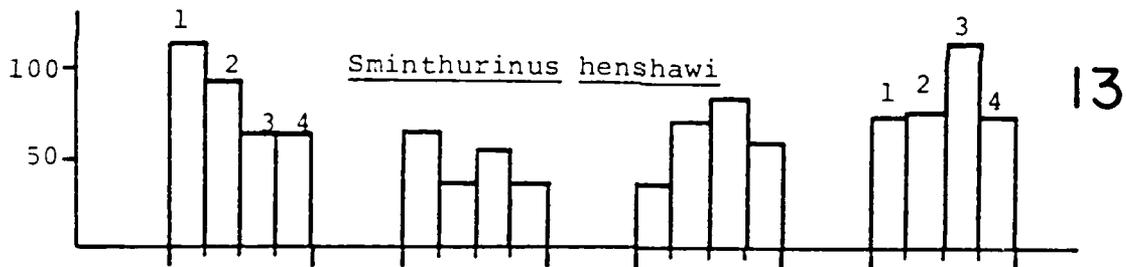
T. flavescens, a large-bodied, scaled entomobryid, was caught less and less frequently over the study period (Fig. 15). Trapping at Turner Rd. yielded consistent (although much smaller) numbers of the species from early August to early September, followed by rapid reduction. Densities/m² of T. flavescens peaked in

Fig. 12. Total number (per 5-day period, summed over all transects), of three dominant Collembola trapped at Silver Lake 1982.

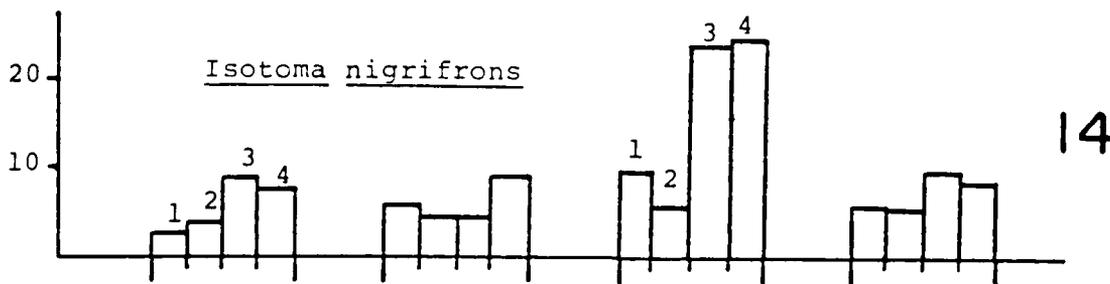
Figs. 13-15. Total number of three collembolans trapped in transects 1-2-3-4, summed over the 5 days in each trapping period.



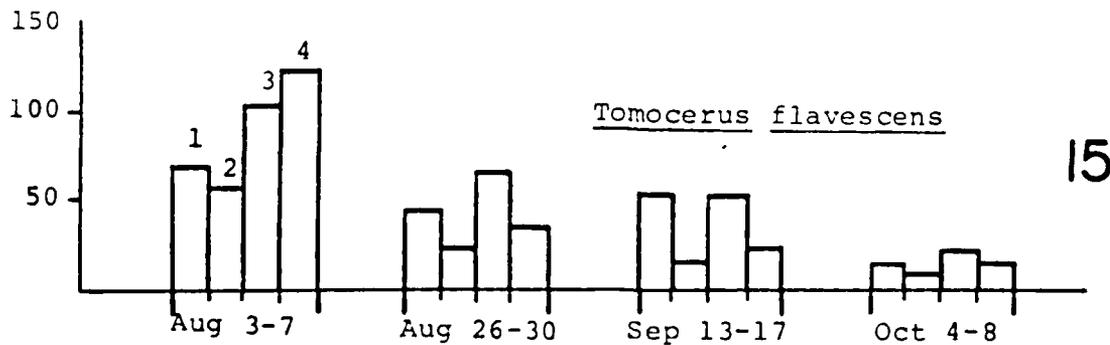
12



13



14



15

August and declined steadily to late October. Decreasing catches at Silver Lake (Fig. 15) therefore seem to be related to a decrease in actual numbers, not activity.

I. nigrifrons, at Turner Rd., exhibited a vertical distribution pattern (refer to Fig. 47) not shared by sminthurids or entomobryids. Its density was high (1000/m² in litter), but the population shifted from litter-dwelling in August to being about equally litter- and soil-dwelling in September - October. Trap catches at Turner Rd. were much lower than at Silver Lake (Fig. 14); activity patterns of I. nigrifrons can therefore not be interpreted with any degree of certainty.

b. Coleoptera:

Carabid beetles made up the bulk of trapped Coleoptera shown in Fig. 8. Three of the four most common species (Fig. 16) exhibited a pronounced decline in late season, while the fourth P. pennsylvanicus, showed an opposite trend.

This pronounced seasonality of occurrence in carabids, illustrated for three common species in Fig. 17-19, has been well-documented (e.g. Barlow 1970). Comparison with Turner Rd. trapping data, which span a full season in 1983 (see Section E), allows the following interpretation: P. melanarius is essentially a mid-summer species; its activity peak was just just barely reflected in the Silver Lake August trap catches (Fig. 17). S. impunctatus (Fig. 18) activity coincides with that of P. melanarius. P. pennsylvanicus, very common in early summer at Turner Rd., disappears for a brief period in late July. Teneral adults reappear in September, and catches reach a peak at the beginning of October. Clearly,

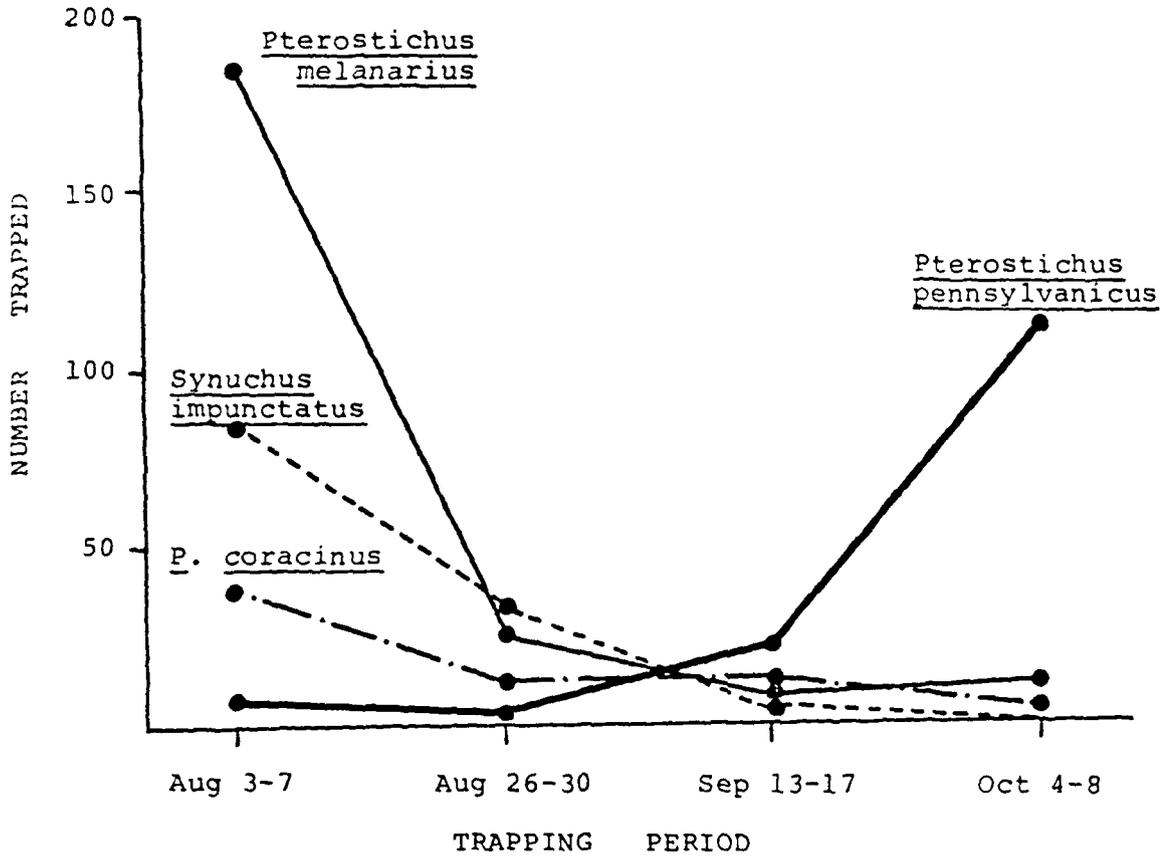
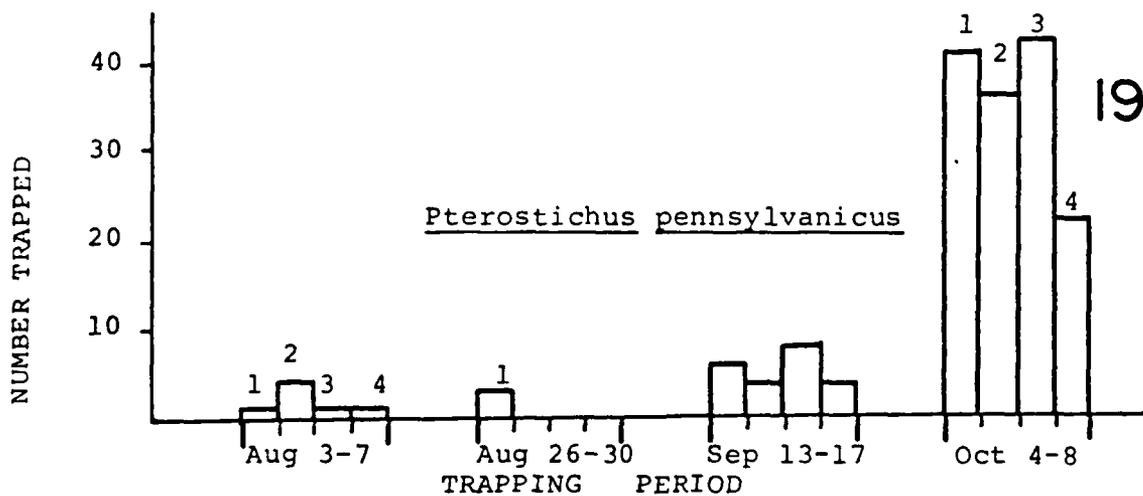
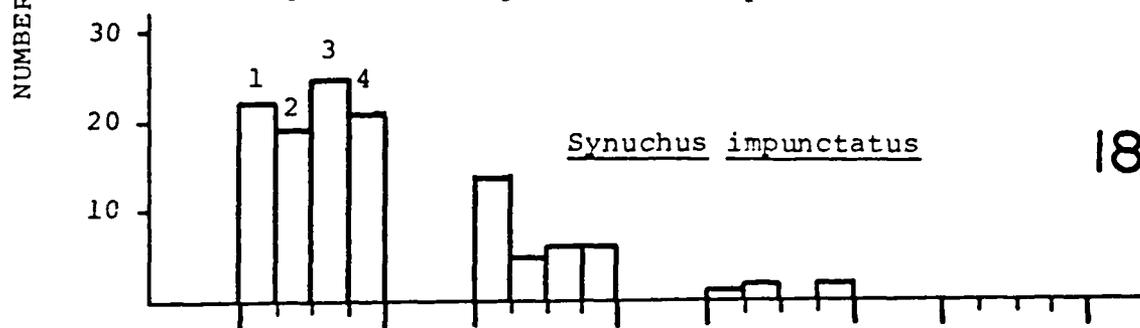
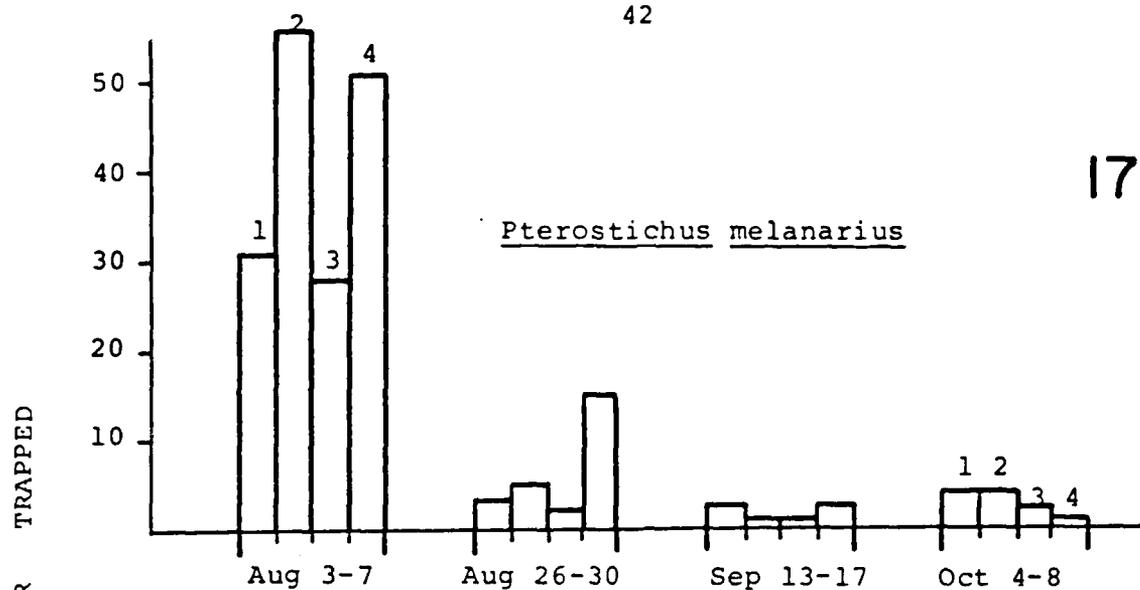


Fig. 16. Total number of four common carabids (summed over all transects and over 5 consecutive days/period) trapped at Silver Lake 1982.



Figs. 17-19. Numbers of three carabid species trapped in transects 1-2-3-4 per trapping period, Silver Lake, 1982.

Silver Lake catches reflect the late-summer decline, and autumn reappearance, of the species.

c. Acarina:

Grouped by suborder (Fig. 20), Mesostigmata and Oribatida both exhibit a late-season decline. Oribatid densities at Turner Rd., much higher in late September than at any other time, can not be used to explain their activity patterns at Silver Lake.

Of 10 mesostigmatid families found in Silver Lake traps, two occurred with reasonable frequency (Fig. 21, 22). Parasitids, in Turner Rd. litter, reach highest densities in September through October. Macrochelidae are much less common. For neither of these, given the present data (including the high between - transect variability), can clear activity patterns be discerned.

Eupodidae and Tydaeidae (Fig. 23, 24) were among the most consistently trapped prostigmatids. For both families, a relationship between densities (at Turner Rd.) and trap catches could be postulated: late-season density increases in Tydaeidae, and decreases in Eupodidae, followed parallel trends in activity at Silver Lake.

d. Opiliones:

L. politum was caught in very low numbers. Data on the other species, of which L. nigripes was the most abundant, are presented in Fig. 25. C. boopis and O. pictus were never prominent. For C. boopis, rare at Turner Rd., there are no supportive data available. O. pictus, however, showed peak occurrence in July, and disappeared during September at Turner Rd. - its decline in Silver Lake traps follows the same trend.

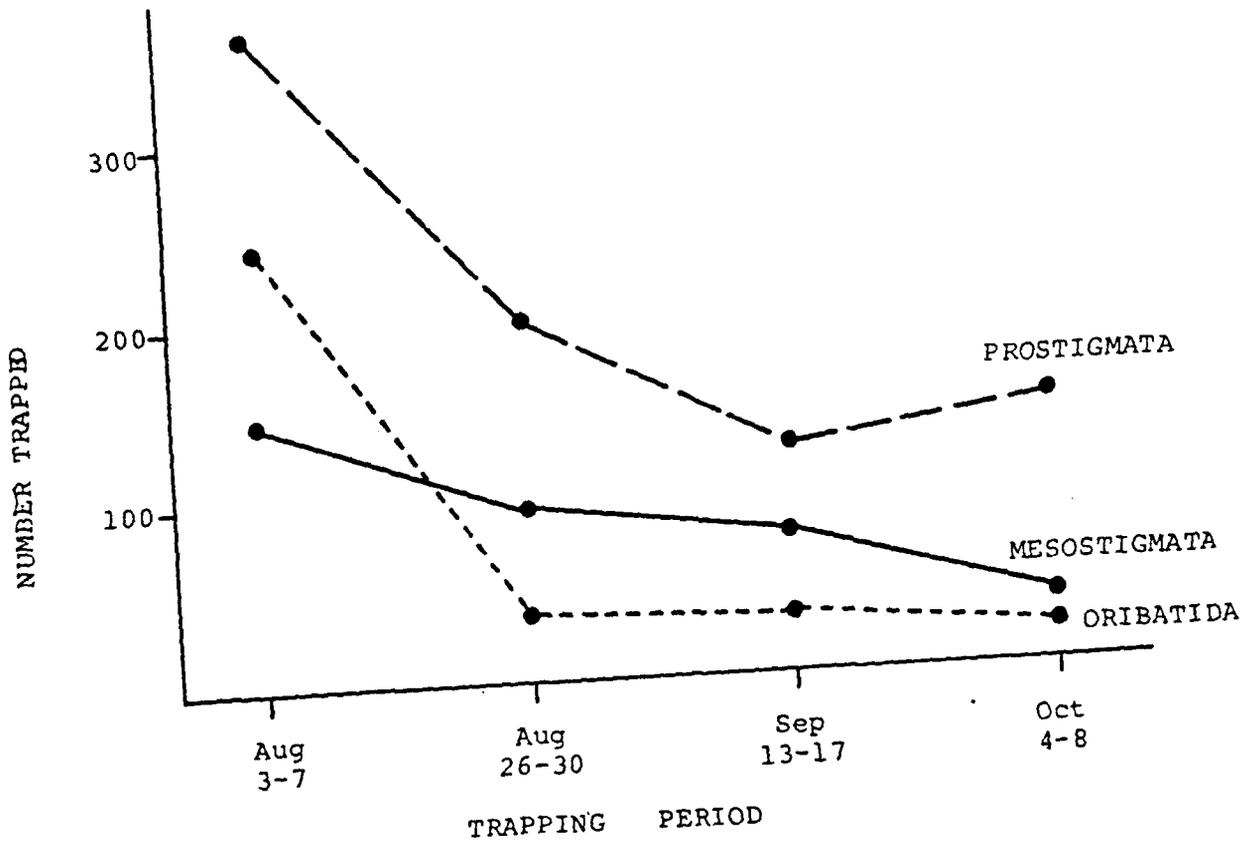
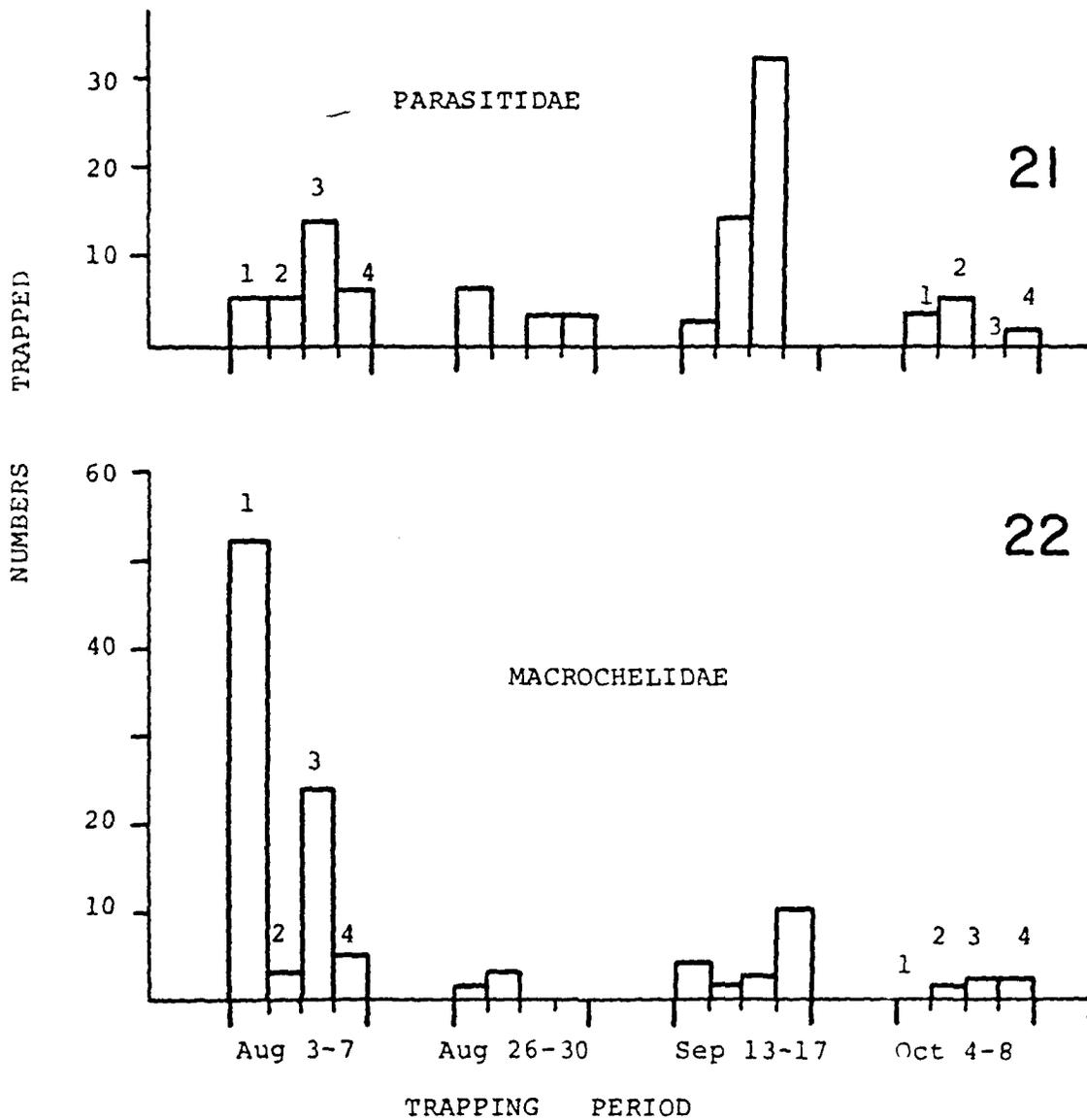
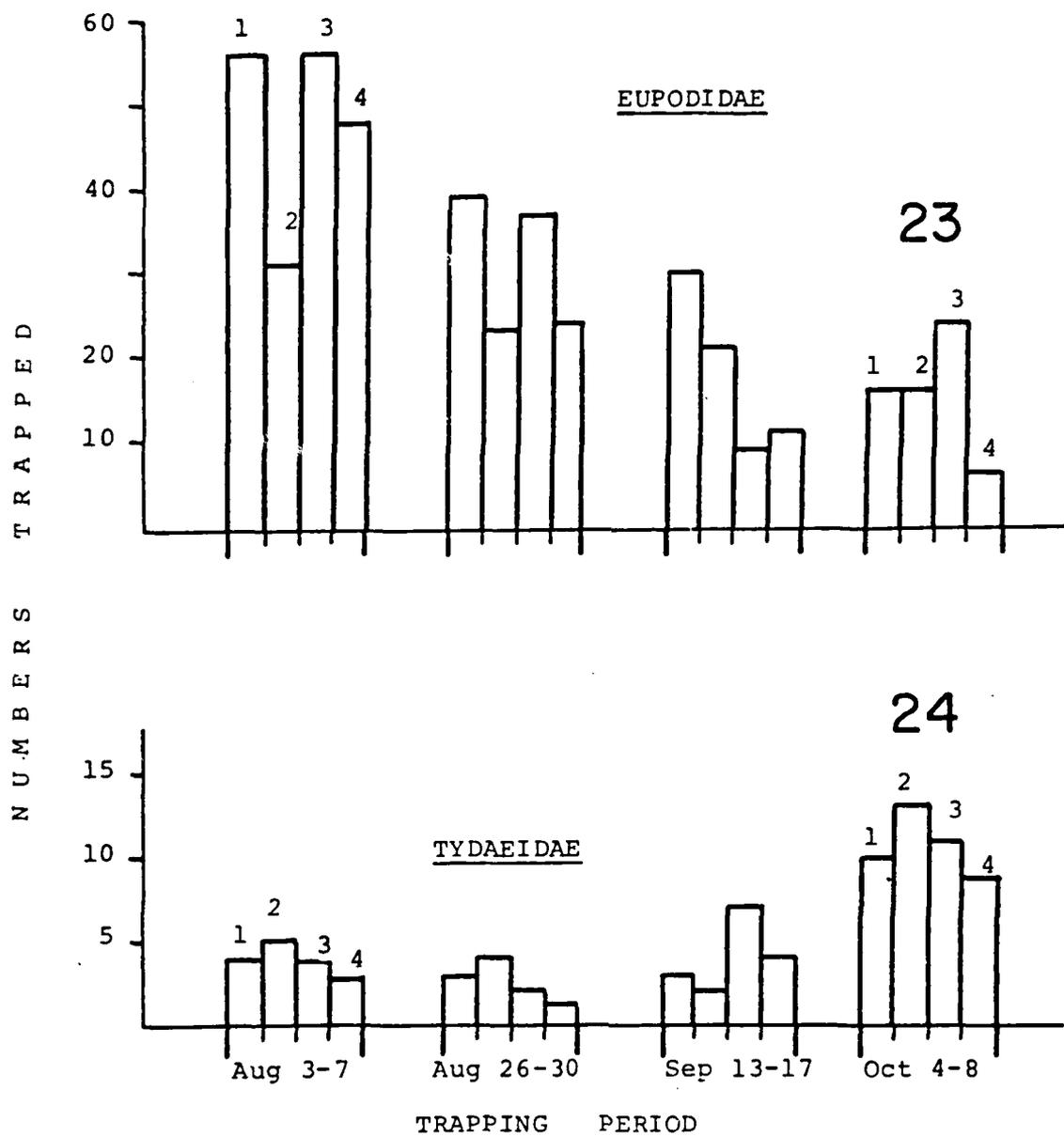


Fig. 20. Number of mites of each suborder trapped per period, summed over all transects, Silver Lake, 1982.



Figs. 21-22. Mesostigmatid families most frequently trapped at Silver Lake, 1982: total numbers in transects 1-2-3-4, summed per trapping period.



Figs. 23-24. Trap catches of common prostigmatids, per 5-day period, in each transect (1-4), Silver Lake 1982.

S. crassipalpe and L. nigripes formed an interesting pair: both had late - season activity peaks (Fig. 26, 27). Their distribution over the four transects, however, provides a case in point concerning possible transect effects: transect 4 caught significantly more S. crassipalpe than other transects; transect 1 caught most individuals of L. nigripes. As pointed out earlier (Collembola totals, Fig. 7), "transect effects" may, for some species or taxa, be more closely related to within - site location than distance between traps. The nature of habitat differences mediating these effects was not obvious and remains unknown.

e. Diplopoda:

Catches illustrated in Fig. 10, consisted almost entirely of U. canadensis. The species provides an example of stage - related activity: only subadults and adults are surface - active. By contrast, peak populations in Turner Rd. litter were composed almost entirely of juveniles, which exhibit practically no horizontal mobility.

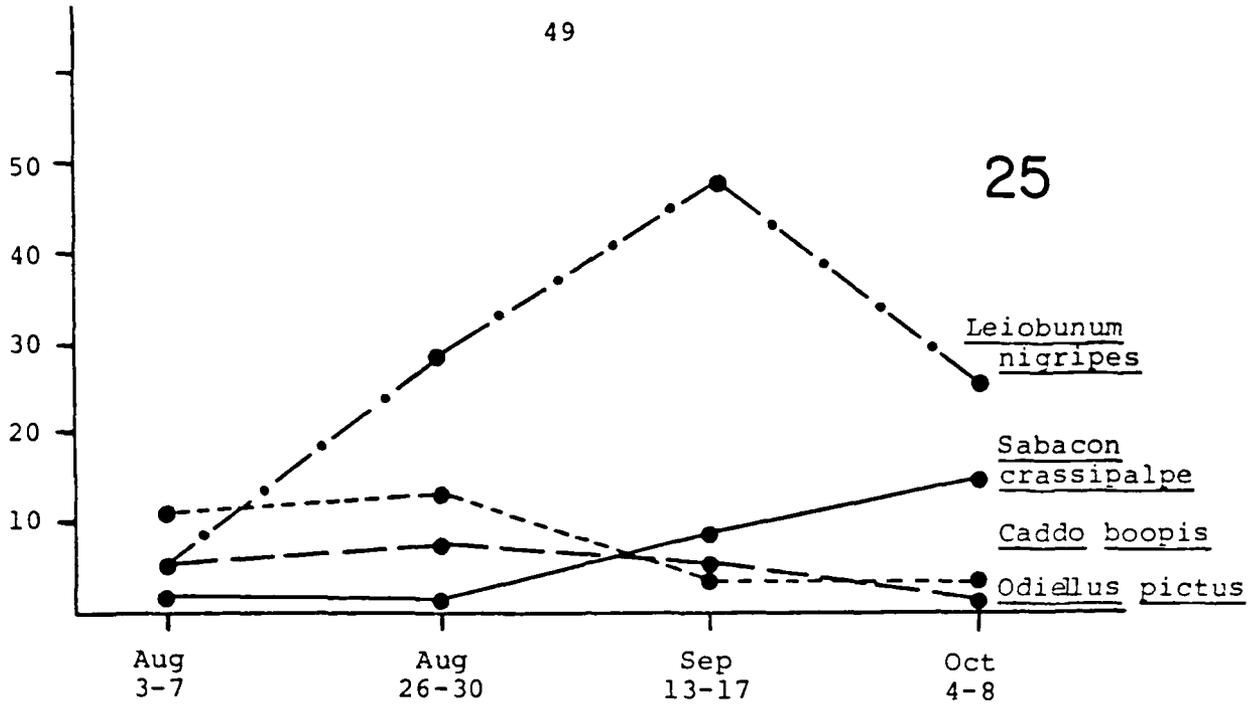
Catches of U. canadensis at Silver Lake were never high, and were negligible at Turner Rd. However, a slight autumnal activity increase in adults and subadults is consistent with reports on juloid activity patterns in a variety of other habitats (Fairhurst 1979; Blower and Gabbutt 1964; Blower and Fairhurst 1968; Blower 1970). In the present study, no consistent transect effects became apparent.

f. Aranei:

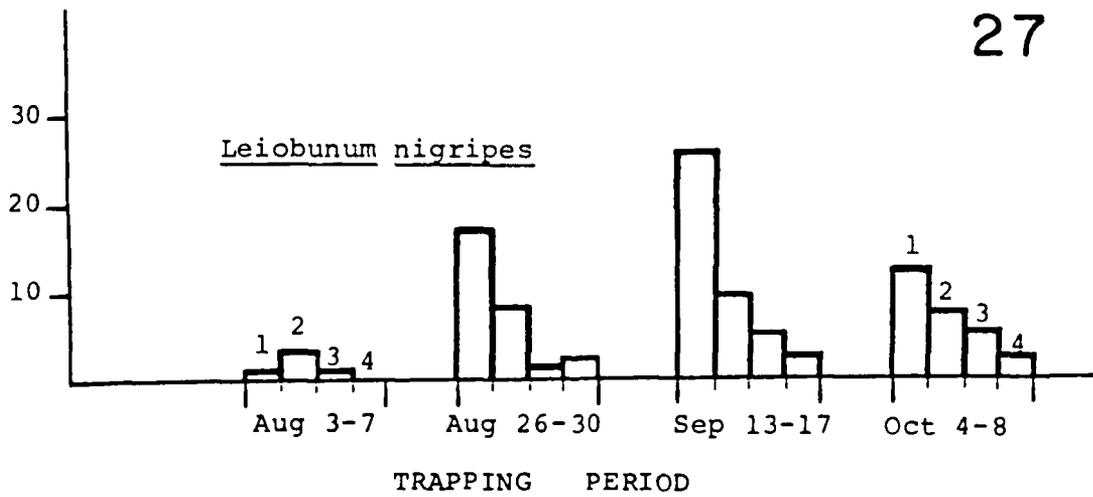
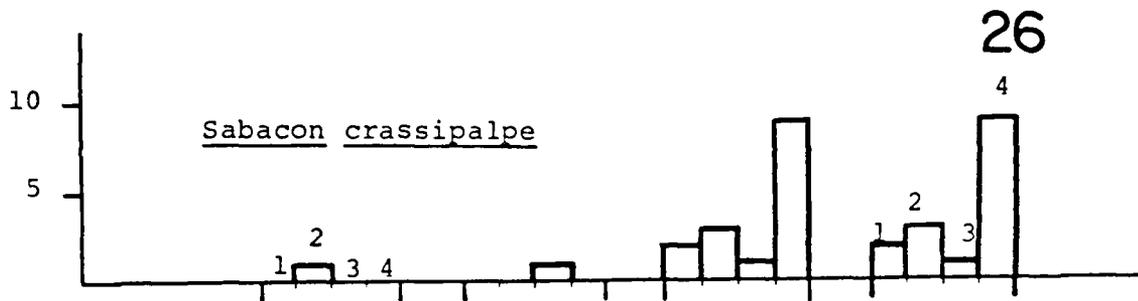
Preponderance of immatures, which could not be identified to species, make interpretation of spider catches tenuous at best.

Fig. 25. Total catches of four opilionid species, summed over all days and all transects, per trapping period, Silver Lake, 1982.

Figs. 26-27. Numbers of S. crassipalpe and L. nigripes caught per transect, summed over 5 days, Silver Lake, 1982.



NUMBERS TRAPPED



TRAPPING PERIOD

Based on total numbers (Fig. 11), no consistent transect effect became apparent.

Several families were represented by single or a few individuals over all dates (e.g. Thomisidae, Amaurobiidae, Philodromidae, Theridiidae, Hahniidae). Adults belonging to four families trapped with regularity are named and listed below:

Lycosidae constituted 26% of the total number trapped (over all dates). Pirata marxi and P. maculatus were relatively common throughout the season.

Linyphiidae (21% of total): Bathyphantes pallida and Centromerus persoluta disappeared from all transects after August 30; C. sylvaticus on the other hand was trapped only in the first week of October.

Agelenidae (20% of total catch) were most heavily represented by the genus Wadotes. Wadotes calcaratus may have been the only species present during the study period; most of these individuals were trapped in the first week of October. Cicurina brevis and Coras montanus both caught in low numbers, showed neither consistent seasonality nor transect - specificity.

Micryphantidae (22% of the total), not further identified, appeared to be equally dominant (relative to other families) in Silver Lake and Turner Rd. catches. Overall, although the two sites share many of the above species, low catches of any one species or genus make any further conclusions esoteric.

In summary, the hypothesis that depletion of surface-active arthropods could be brought about by intensive trapping along linear transects can not be confirmed, given the conditions of this experiment. Some generalizations are possible, keeping in mind that

trapping was done only late in the season, and referring to phenological patterns discussed earlier:

a) full-season trapping might have measureably depleted some populations, by removing reproductive adults from spring-summer populations, thereby reducing the numbers of their offspring available for trapping in the fall (e.g. certain Coleoptera).

b) depletion of arthropod populations by trapping is potentially more severe for species with relatively constant populations over the season. Those that exhibit temporally brief and intense population peaks are not available for a long enough period of time (e.g. Opiliones). Their maturation/activity minima cannot be distinguished from trapping effects.

c) in general, Silver Lake data validate the experimental design we adopted in 1983 for Test and Control sites. Although a "grid effect" potentially exists, distance between any two traps is 10 m (greater than those reported by most other researchers, and much greater than the 4 m separating traps in Silver Lake transect 1). In addition, quadrats with traps (Fig.3) are not all contiguous, i.e. the grid is "broken" by check plots and rejected quadrats, so that effective distance between traps is often ≥ 20 m.

D. Soil-litter Arthropoda, 1982

1. Introduction

Data discussed in the following sections stem from the 1982 field season (August to October), i.e. from the Turner Rd. site. They have proven invaluable as a descriptive introduction to the nature and activity of arthropod populations in the ELF area. Indeed, (refer to Table 7), many of the species found in definitive sites also abound at Turner Rd. Their co-occurrences alone justify the effort of presenting these pre-construction data sets, even if the site they stem from has now been relinquished.

2. Methods

Soil samples: cylindrical core samples (5.2 cm diameter) were taken to a depth of 15 cm, stored in plastic bags, and transported to the field laboratory. Sampling dates were spaced 14 days apart, 20 replicate (random) samples being taken per date.

Litter samples: at intervals of 14 days, 20 samples of litter, including woody debris, were taken in the site. Samples were 25 x 25 cm square, obtained by cutting through the litter along the inside periphery of a metal frame, then transferring litter and debris to plastic bags. In this study, "litter" includes partly decomposed or comminuted leaf aggregates which are easily gathered by a brushing motion of the hand.

In 1982, the 20 samples taken per date were distributed as 10/10 over two types of locations, termed "Hill" and "Depression". Because of the small-scale topographic heterogeneity of the site, it seemed advisable to obtain a preliminary estimate of within-site

variability in arthropod densities related to topography. The two types of location differed mainly in extent (depth) of litter cover, although Hill locations were never found to be completely denuded of leaf litter.

All samples were transported in coolers to the field laboratory, and extracted over 4-7 days in modified Tullgren funnels. Extracted animals were stored in 95% ethanol plus 1% glycerine.

3. Results

3.1. Abundance and seasonality of major taxa

All arthropods were initially identified to order. Some groups will not be discussed in this report, for three reasons: a) Tullgren extraction is an inappropriate method for assessing their densities (e.g. molluscs, ants, enchytraeid worms); b) they can not be considered true litter/soil dwellers (e.g. Hymenoptera, Diptera, Lepidoptera) and c) they were extracted infrequently and in low numbers (e.g. Homoptera, Neuroptera larvae, opilionids). Specimens of these groups were placed in storage, and are thus available should they become needed to answer questions arising in the future.

In Table 11, major taxa of the Turner Rd. arthropod assemblage are listed, and peak densities are given for each. Standard errors are very large, indicating the clumped distribution typical of soil-litter arthropods. Mites and Collembola are numerically dominant; a few taxa, e.g. Protura, spiders, pseudoscorpions, show distinct preference for either litter or soil.

Table 11. Peak densities (number/m² + S.E.) of arthropods in soil and litter, Turner Rd., 1982. In parentheses: month of peak occurrence.

Taxon	Peak densities/m ² + SE (month)	
	Soil	Litter
Diplopoda	+	24.0 ± 5.5(Sept.)
Chilopoda	325 ± 90(Oct.)	54.4 ± 12.7(Aug.)
Protura	175 ± 75(Sept.)	+
Collembola	5850 ± 1474(Sept.)	1558 ± 416(Aug.)
Coleoptera	125 ± 50(Aug.)	53.6 ± 9.4(Sept.)
Coleoptera larvae	600 ± 180(Oct.)	50.4 ± 9.8(Aug.)
Diptera larvae	1350 ± 800(Aug.)	97.6 ± 19.2(Sept.)
Lepidopt. larvae	-	8.0 ± 3.2(Sept.)
Psocoptera	150 ± 65(Sept.)	16.0 ± 4.6(Aug.)
Thysanoptera	+	14.4 ± 8.8(Aug.)
Acari		
Oribatida	3525 ± 836(Sept.)	2262 ± 371(Sept.)
Mesostigmata	1400 ± 387(Oct.)	493 ± 95(Sept.)
Prostigmata	4525 ± 1090(Oct.)	2858 ± 792(Sept.)
Astigmata	+	31.2 ± 17.1(Sept.)
Aranei	+	92.0 ± 10.0(Sept.)
Pseudoscorpiones	+	40.0 ± 11.6(Sept.)

Many groups reached peak densities in September. It is likely that spring peaks occur as well - 1983 data, not available at this time, will document early-season population dynamics. Based on data for the 1982 half-season, it appears that the majority of arthropod groups, though not all, increase in numbers in early to late fall. In Figures 28 to 34, seasonal density fluctuations are illustrated for numerically or functionally (e.g. predators) important groups. In the case of mites (Fig. 28 and 29), vertical redistribution in late fall becomes obvious for all suborders. Seasonal stratification has been reported by others (e.g. Luxton 1981, 1982). It is likely, however, that species or species groups within higher taxa differ greatly in their habitat preferences and their responses to environmental change. In subsequent sections, more detailed analyses will therefore be presented.

3.2. Horizontal distribution patterns:

Hill vs. Depression

Density data discussed in the preceding section were based on combined hill and depression samples, and therefore represent arthropod population estimates for the site as a whole. As might be expected, accumulated litter in depressions supports greater numbers of detritivores as well as of predators. As a point of interest, the magnitude of this effect was quantified for selected taxa, and is illustrated in Figures 35 to 40, using average numbers of animals per sample. Differences were generally most pronounced in August, least so in late September and October.

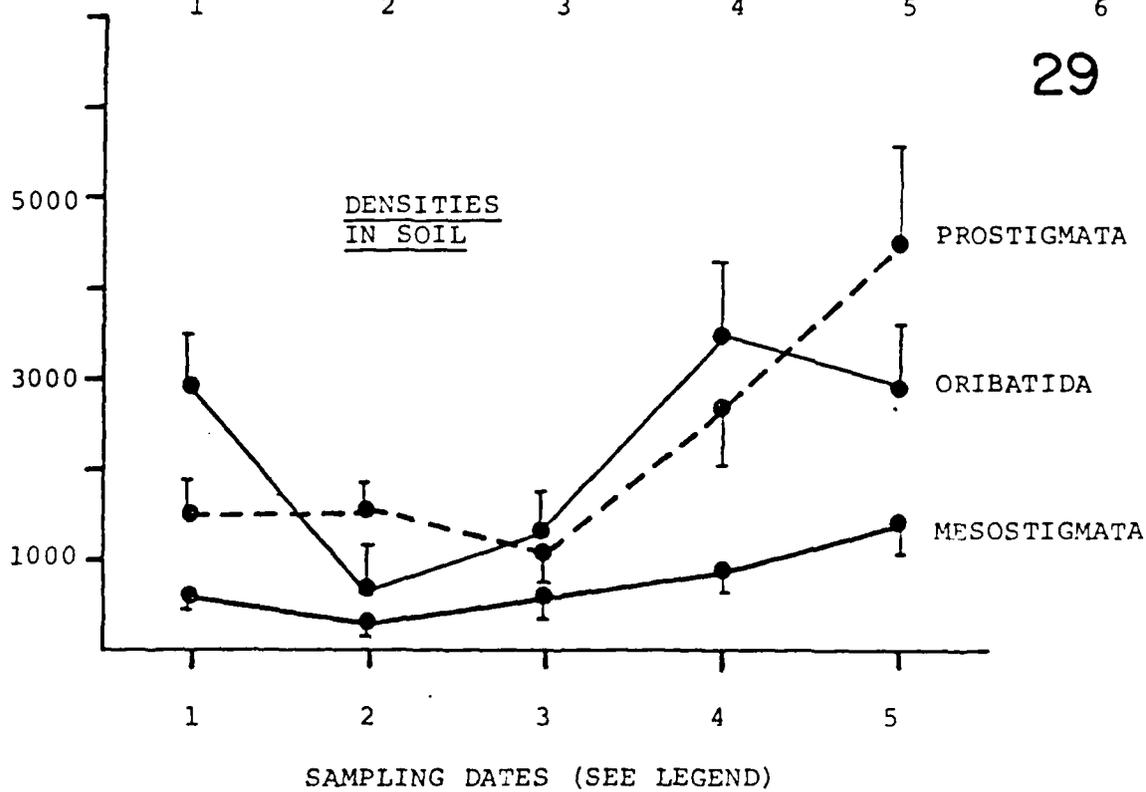
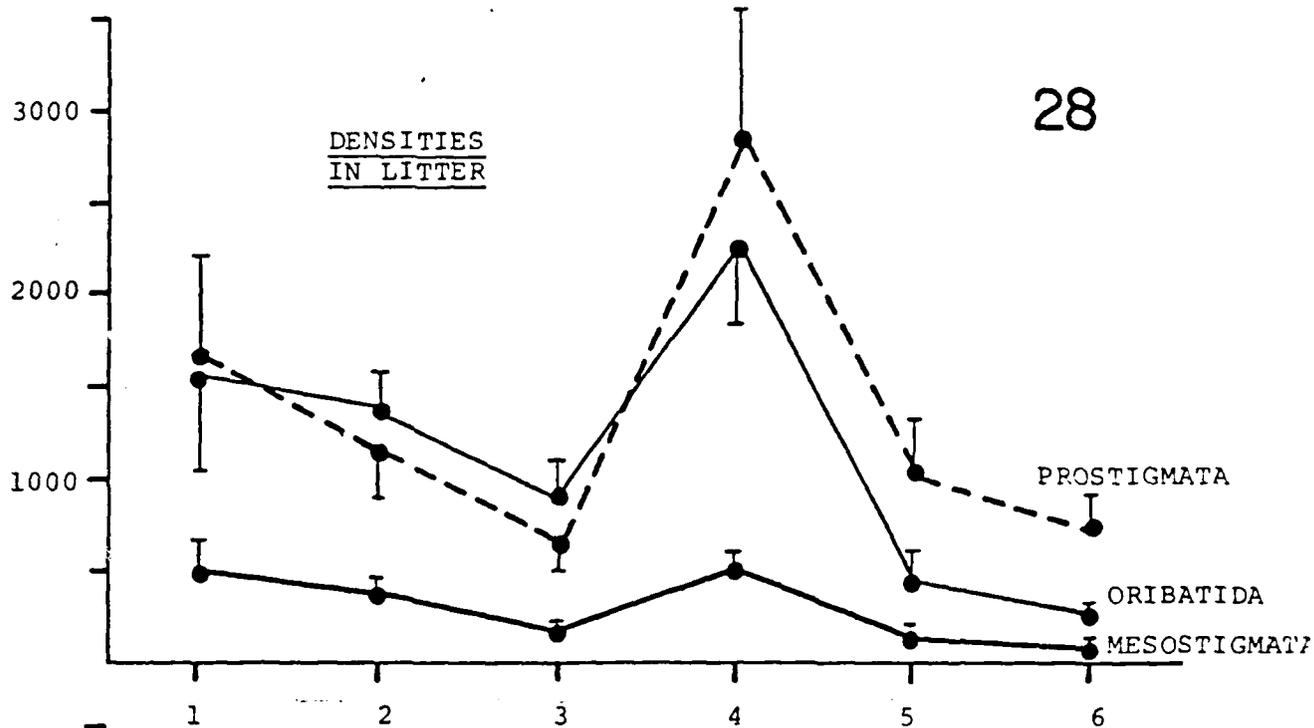
That population size of arthropods varies greatly in space is well known (e.g., Stebayeva 1975; Dynger 1978; Snider 1984;

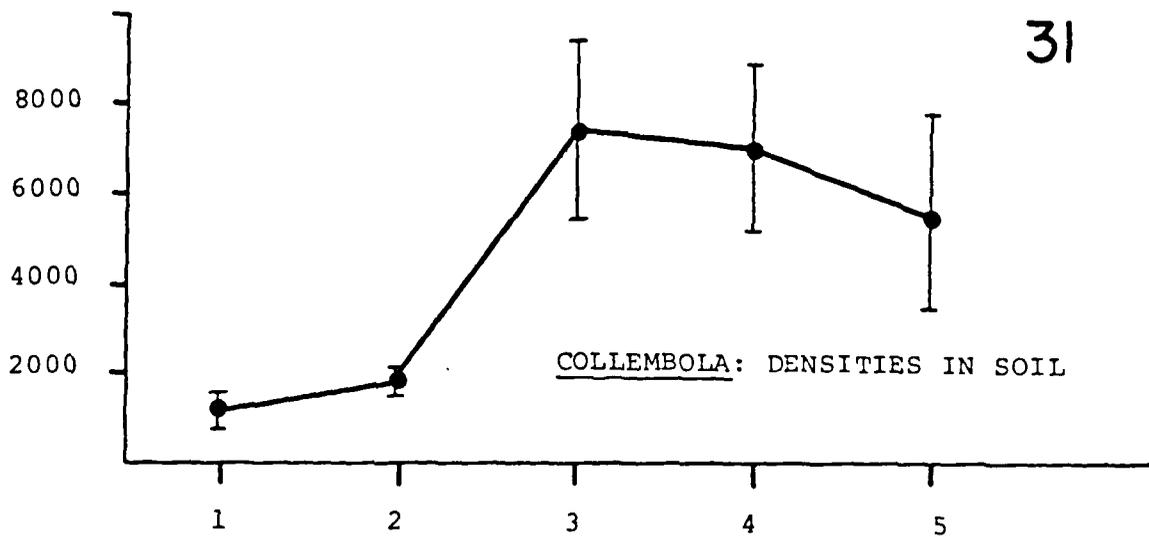
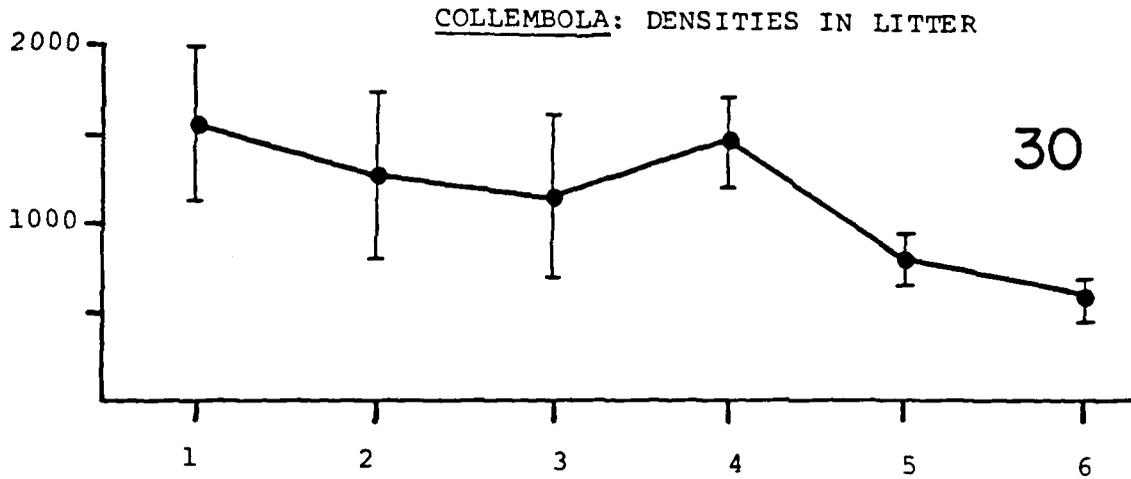
Figs. 28-34. Densities / m² ± SE of arthropods in soil and litter (n = 20 samples/date), Turner Rd 1982.

Key to dates (1 through 6):

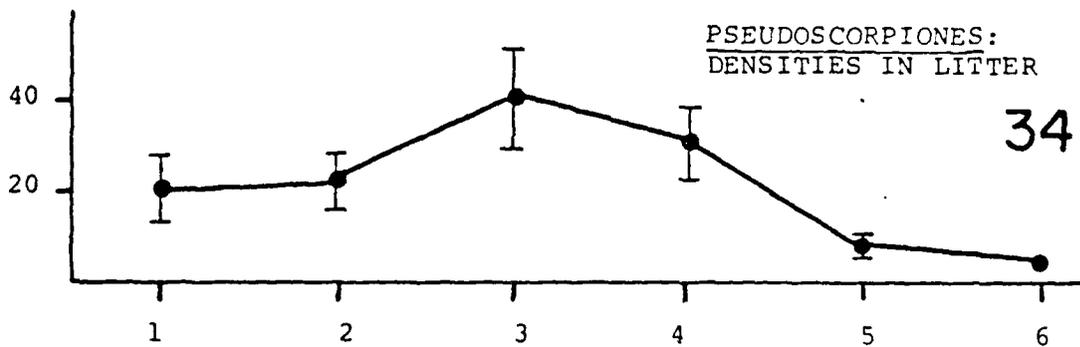
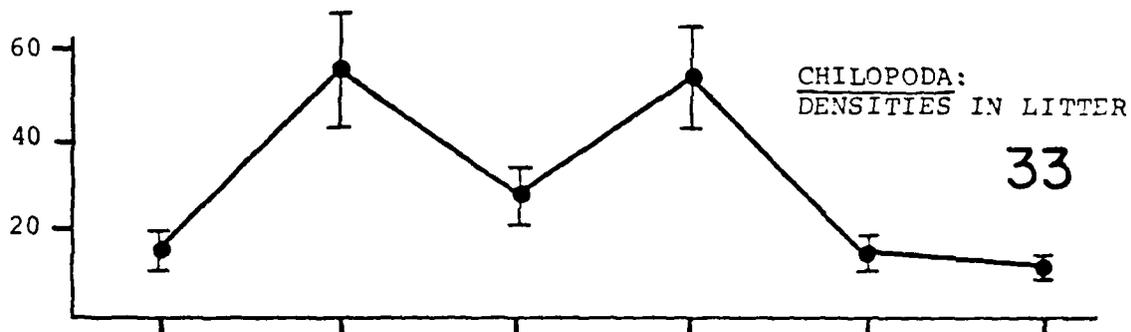
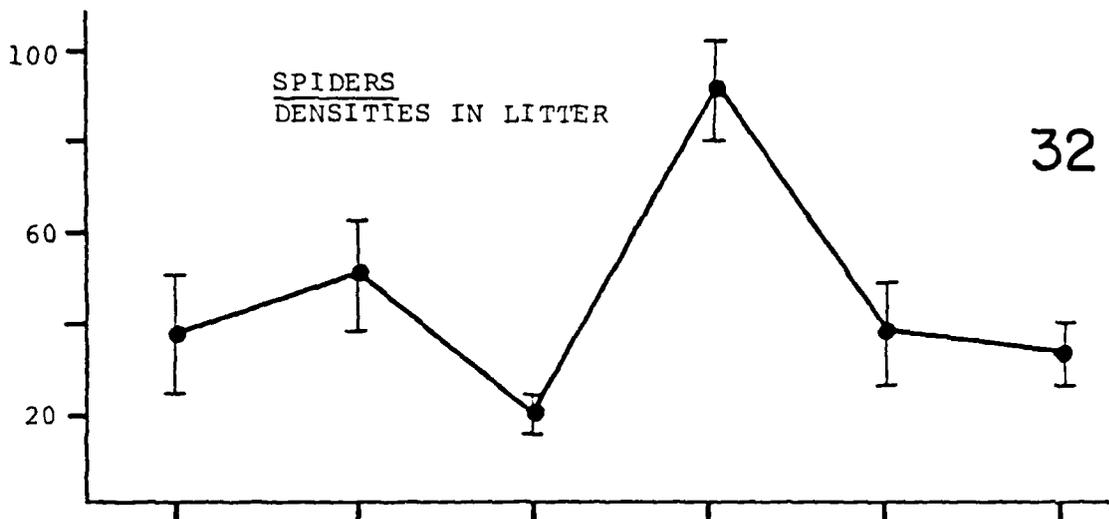
- 1 - August 10+12 (litter), August 16 (soil);
- 2 - August 25+27 (litter), August 30 (soil);
- 3 - September 7+9 (litter), September 14 (soil);
- 4 - September 22 (litter), September 28 (soil);
- 5 - October 4 (litter), October 11 (soil);
- 6 - October 22 (litter only).

For reasons of clarity, soil and litter data from samples taken 4 to 6 days apart are lumped as one date in Figs. 28-34.



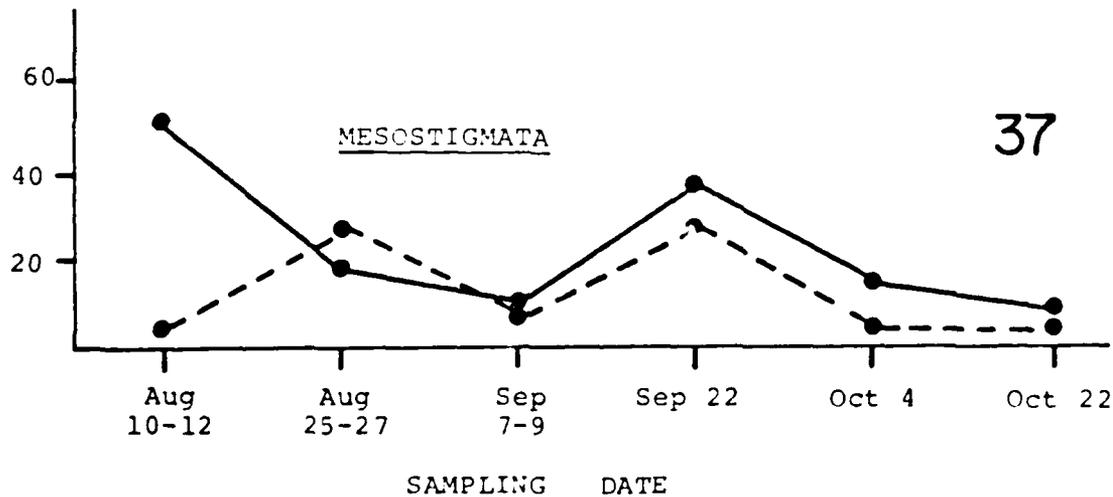
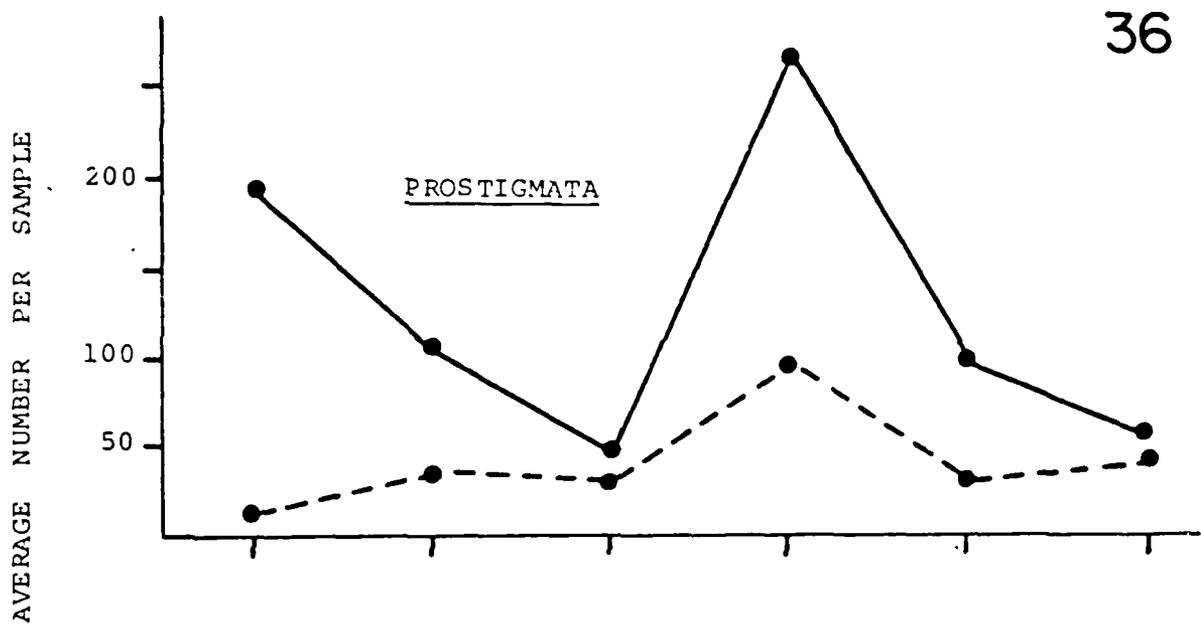
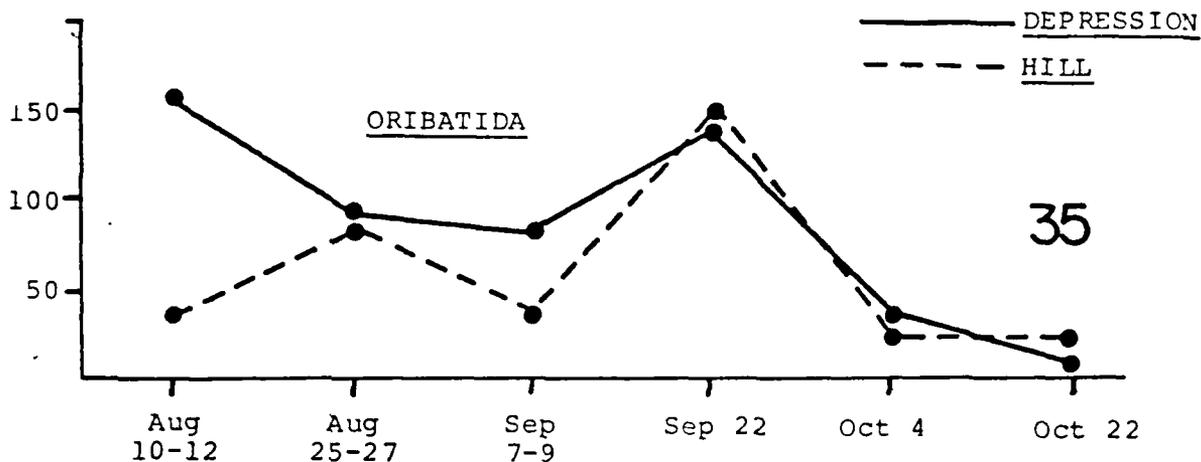


SAMPLING DATES (SEE LEGEND)

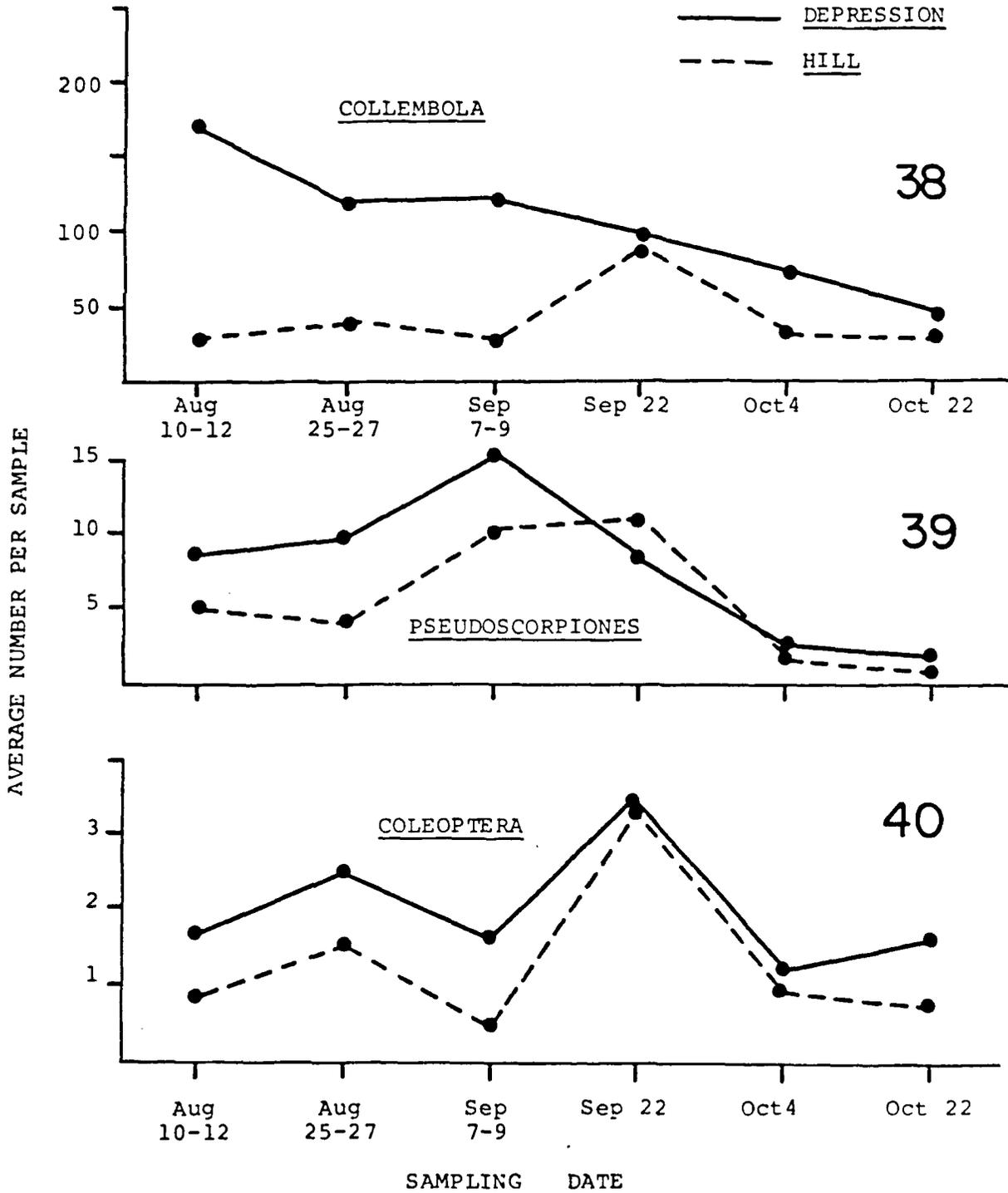


SAMPLING DATES (SEE LEGEND)

Figs. 35-40. Average number/sample (SE omitted) of selected arthropod taxa extracted from Hill vs. Depression samples of litter (n = 10 samples per location), Turner Rd 1982.



SAMPLING DATE



Usher 1976). At Turner Rd., the non-significant differences between means on September 22 (exception: Prostigmata) may have been due to rapidly falling temperatures (Fig. 41): litter-rich depressions generally remain cooler than high areas throughout the summer (Snider 1984), but reductions in air temperature would make this difference "biologically non-significant" for arthropods. Other major environmental parameters do not offer any further explanation. Prior to September 22, litterfall was insignificant, i.e., no addition to the thin litter cover of hills could have taken place. Furthermore, rainfall was plentiful prior to both the early August and the late September sampling dates (Fig. 41).

Although no standard errors are shown in Figs. 35 to 40, it is clear from data and illustrations presented in later sections that arthropod aggregation tendencies constitute the major source of sample variance. In 1982, by sampling different within-site locations, we introduced a second source of variation, of great magnitude in some taxa (e.g. Prostigmata, Collembola), but relatively insignificant in others. Only by comparing seasonal data from 1982 to 1983 will we be able to quantify ensuing differences in population estimates. In general, we may conclude that random sampling, as performed in all sites in 1983, will yield valid estimates of arthropod communities characteristic of each site.

3.3. Vertical distribution and abundance in litter and soil

Some groups of arthropods respond to changing environmental conditions, be they temperature, moisture, or food resources, by horizontal or vertical migration. Those which are adapted to a specific stratum of the forest floor system, however, are distinguishable from mobile forms by consistent absence from one or

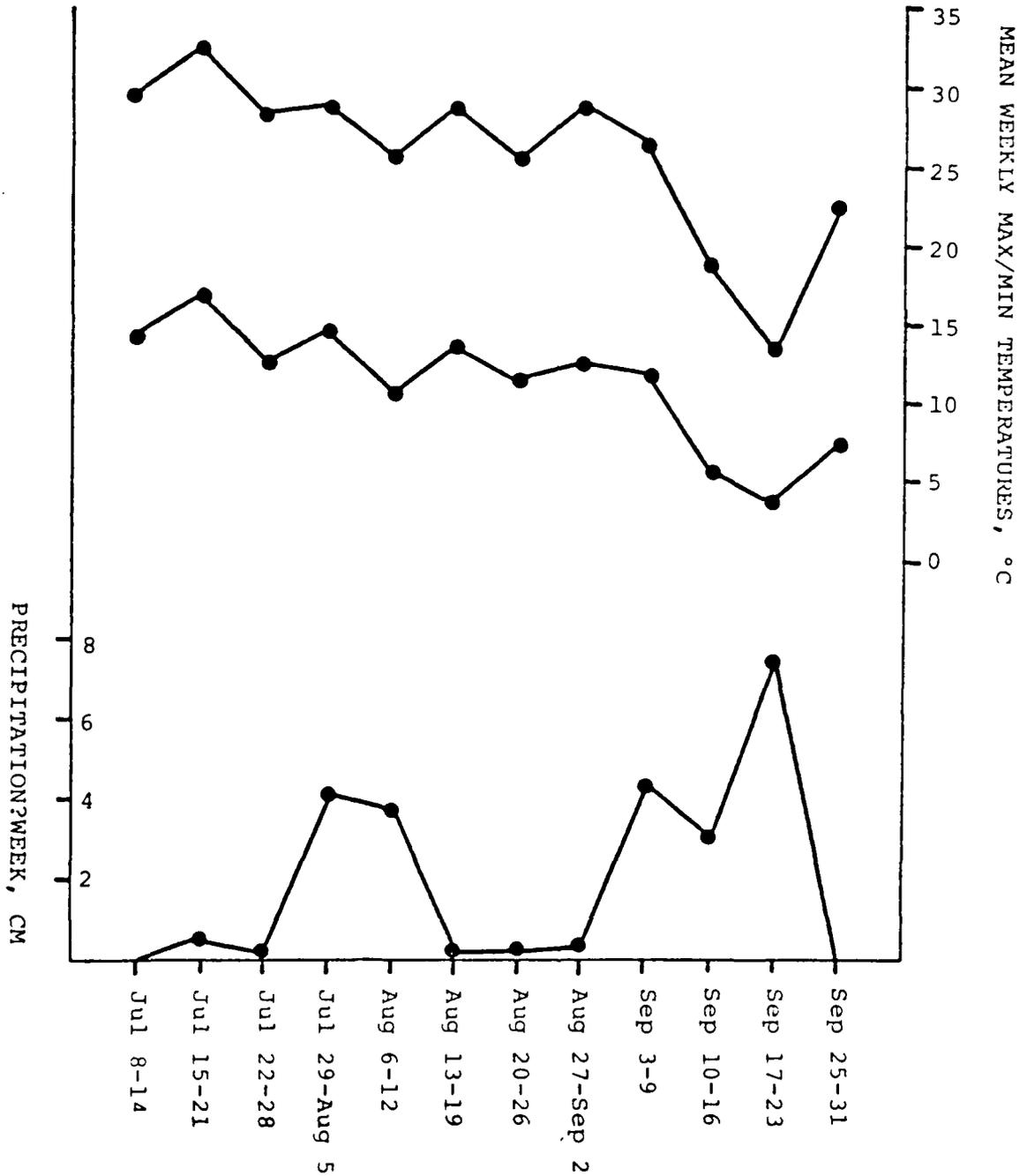


Fig. 41. Total precipitation/week, and weekly averages of max/min temperatures, 1982: records from NOAA weather station at Crystal Falls, approx. 24 km distant from Turner Rd.

the other microhabitat. These specialized forms would thus show constancy of vertical distribution patterns, apparently unrelated to climatic changes.

In the following, seasonal trends in abundance and distribution of selected species populations are described and discussed.

a. Collembola:

Gisin (1943) defined different collembolan "life forms" based on the relationship between morphological characteristics and depth distribution. Surface-inhabitants would thus possess well-developed eyes, furca, and pigmentation, while deeper-living species would tend to be small, colourless and blind. A recent publication by Hagvar (1983) illustrates and confirms these relationships through detailed quantification of species-specific depth distribution.

Collembola associations at Turner Rd. were found to be relatively diverse (Table 12), and overall vertical distribution tendencies (litter vs. soil) conformed well to Gisin's life form categories. Peak densities given in Table 12 indicate, as expected, that Onychiuridae generally are euedaphic, while Sminthuridae and Entomobryidae are litter-inhabitants. On the other hand, relative distributions apparent in Table 12 also point to the existence of different degrees of flexibility: isotomids tend to have a wider range in vertical distribution than some of the scaled, strictly hemiedaphic entomobryids. Keeping in mind that our data span only the latter part of the season, species-specific densities and distributions, as discussed below, yield a preliminary description of the Collembola association at Turner Rd.

Table 12. List of species, density \pm SE, and dominance (%) indices for species within each family of Collembola, Turner Rj, 1982.
 (- absent; + present in frequencies <20%; total number/family given in parentheses).

	Peak densities/m ² + S.E. (month)		
	D%	SOIL	LITTER
<u>Neelidae (137)</u>			
<u>Neelus minimus</u>	67.9	70.0 \pm 25.0(Sept.)	25.6 \pm 9.6(Aug.)
<u>N. tristani</u>	21.2	+	8.0 \pm 2.7(Aug.)
<u>N. snideri</u>	10.2	+	4.0 \pm 1.6(Aug.)
<u>N. minutus</u>	0.7	-	+
<u>Sminthuridae (246)</u>			
<u>Sminthurinus henshawi</u>	90.2	+	64.0 \pm 13.5(Aug.)
<u>Sminthurides lepus</u>	3.3	-	+
<u>Bourletiella hortensis</u>	1.6	-	+
<u>Dicyrtoma marmorata</u>	0.8	-	+
<u>Sminthurus butcheri</u>	0.4	-	+
<u>Arrhopalites anarus</u>	2.9	-	+
<u>A. benitus</u>	0.8	-	+
<u>Entombryidae (838)</u>			
<u>Tomocerus flavescens</u>	23.8	+	59.2 \pm 19.8(Aug.)
<u>Lepidocyrtus paradoxus</u>	54.5	2950 \pm 1046(Sept.)	80.8 \pm 1.6(Oct.)
<u>L. helenae</u>	6.1	-	21.6 \pm 6(Sept.)
<u>L. violaceus</u>	0.6	-	+
<u>L. unifasciatus</u>	0.1	-	+
<u>Entombrya comparata</u>	9.8	+	21.6 \pm 6.8(Sept.)
<u>E. assuta</u>	0.6	-	+
<u>E. multifasciata</u>	0.1	+	-
<u>Orchesella hexfasciata</u>	4.4	-	15.2 \pm 5.9(Aug.)
<u>Isotomidae (6465)</u>			
<u>Isotoma nigrifrons</u>	76.7	675 \pm 224(Sept.)	1021 \pm 355(Aug.)
<u>I. notabilis</u>	0.7	+	+
<u>I. viridis</u>	0.02	-	+
<u>Folsomia nivalis</u>	16.6	1325 \pm 746(Sept.)	266 \pm 75(Sept.)
<u>Proisotoma minima</u>	5.6	+	277 \pm 267(Sept.)
<u>Isotomiella minor</u>	0.02	+	-
<u>Anurophorus binoculatus</u>	0.4	-	+
<u>Onychiuridae (424)</u>			
<u>Tullbergia granulata</u>	30.7	775 \pm 272(Sep.)	10.4 \pm 5.3(Aug.)
<u>T. mala</u>	22.6	775 \pm 203(Sep.)	+
<u>Onychiurus similis</u>	11.2	375 \pm 210(Oct.)	+
<u>O. affinis</u>	24.8	+	39.2 \pm 14.7(Sep.)
<u>O. parvicornis</u>	10.6	-	19.2 \pm 13.0(Aug.)
<u>Hypogastruridae (648)</u>			
<u>Willemia intermedia</u>	6.8	+	14.4 \pm 11.2(Sep.)
<u>W. similis</u>	0.3	+	+
<u>W. denisi</u>	4.9	-	19.2 \pm 10.5(Sep.)
<u>Friesea sublinis</u>	0.6	+	+
<u>Anurida furcifera</u>	1.2	+	+
<u>A. pygmaea</u>	1.7	-	+
<u>Neanura muscorum</u>	4.3	-	9.6 \pm 4.1(Aug.)
<u>Odontella sp.</u>	67.0	-	142 \pm 64(Sep.)
<u>Pseudachorutes A</u>	2.6	-	+
<u>Pseudachorutes B</u>	8.6	-	20.0 \pm 6.2(Sep.)
<u>Pseudachorutes C</u>	1.9	-	+

Neelidae (Fig. 42) are represented by four species, of which N. minutus is rare, and N. minimus dominant (dominance index 68%). They inhabit both soil and litter, but their frequency in soil samples is low. Fig. 42 shows estimated densities for the total of all species. Neelid densities in soil, because of low frequencies, will need to be confirmed through 1983 data. However, our data agree with those given by Hagvar (1983), who obtained distinct maxima of N. minimus at depths of 3-6 cm in the soil.

Sainthuridae (Fig. 43), of which S. henshawi is overwhelmingly dominant (90%), are clearly hemiedaphic. S. henshawi reaches densities of 50-60 individuals/m², and appears to be strongly seasonal.

Five species of Entomobryidae constitute the bulk of this family. Morphologically, all would be classified as hemiedaphic. With the exception of L. paradoxus (Fig. 44), they are found to frequent litter only (Figs. 45 and 46). In the case of L. paradoxus, the staggering density peak of close to 3000/m² (± 1046 S.E., thus highly aggregated) in soil is clearly an artifact: the species is morphologically hemiedaphic (scaled, darkly pigmented, with long antennae, legs and furcula), and behaviorally tends to be not only hemiedaphic, but epigeic: in meadows, it climbs vegetation in bright sunlight. L. paradoxus is thus likely to be a dominant, highly aggregated entomobryid. We suspect, however, that under the conditions in the wooded Turner Rd site, it frequents the surface of the soil and the coarse cervices available there. Additional, more finely stratified sampling is needed to confirm these suggestions.

T. flavescens (dominance 24%) shows a steady decline in numbers over time (Fig. 45), in contrast to observations published by Huhta and Mikkonen (1982). These authors noted a pronounced density

increase of the species in the fall. It is possible that the number and timing of reproductive periods, as determined by climatic conditions, result in widely discrepant seasonal densities (Joose 1969).

Both L. helenae and E. comparata show September density peaks (Fig. 6). Whether these species are univoltine, as many of their congeners appear to be in Scandinavian countries (Persson and Lohm 1977; Huhta and Mikkonen 1982), will become apparent from samples obtained in spring and summer 1983.

I. nigrifrons (dominance 77%) and F. nivalis (dominance 16%) both portray the flexibility of "intermediate" life forms (Fig. 47 and 48). They frequent litter as well as soil, and I. nigrifrons in particular reaches very high densities. Both species tend to migrate into the soil in the fall, probably under the influence of decreasing temperature (e.g. Usher 1970; Marshall 1974; Hale 1966).

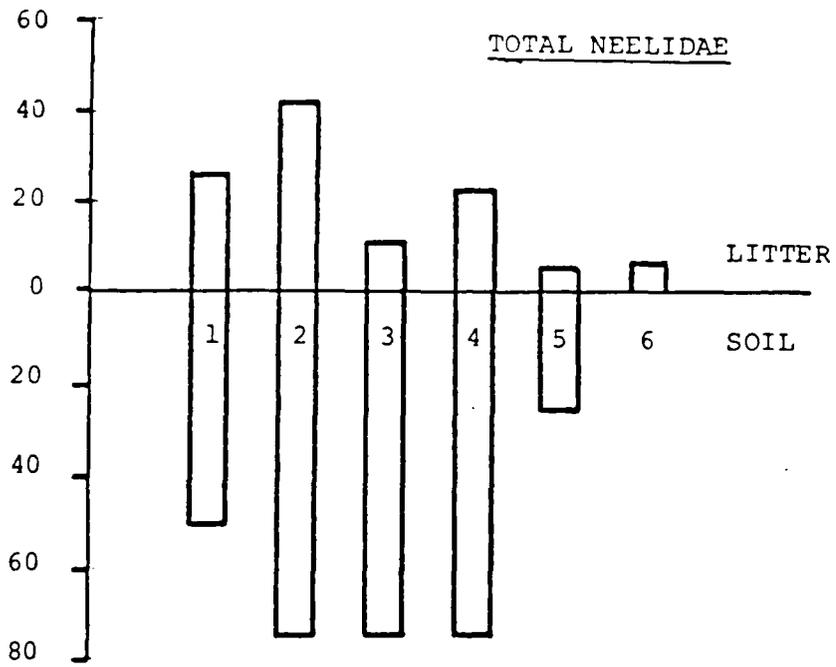
Members of the family Onychiuridae are typically euedaphic (Gisin 1943), especially the small, spindle - shaped genus Tullbergia. At Turner Rd, both species of Tullbergia, (Fig. 49) and O. similis (Fig. 50) are essentially confined to the soil and occur in high densities which peak late in the season. Unexpectedly, O. parvicornis and O. affinis were essentially absent from soil samples (Table 12, Fig. 51). Conditions in the litter, at least during the latter part of 1982, allowed them to remain in that stratum; it is highly suggestive, in fact, that 100% of O. parvicornis, and 90% of O. affinis specimens were extracted from "depression" samples. These morphologically euedaphic onychiurids may thus be more opportunistic than other members of the family. Other authors have reported

Figs. 42 - 52. Vertical distribution of common Collembola species in litter and soil, Turner Rd 1982: seasonal densities / m² (SE omitted).

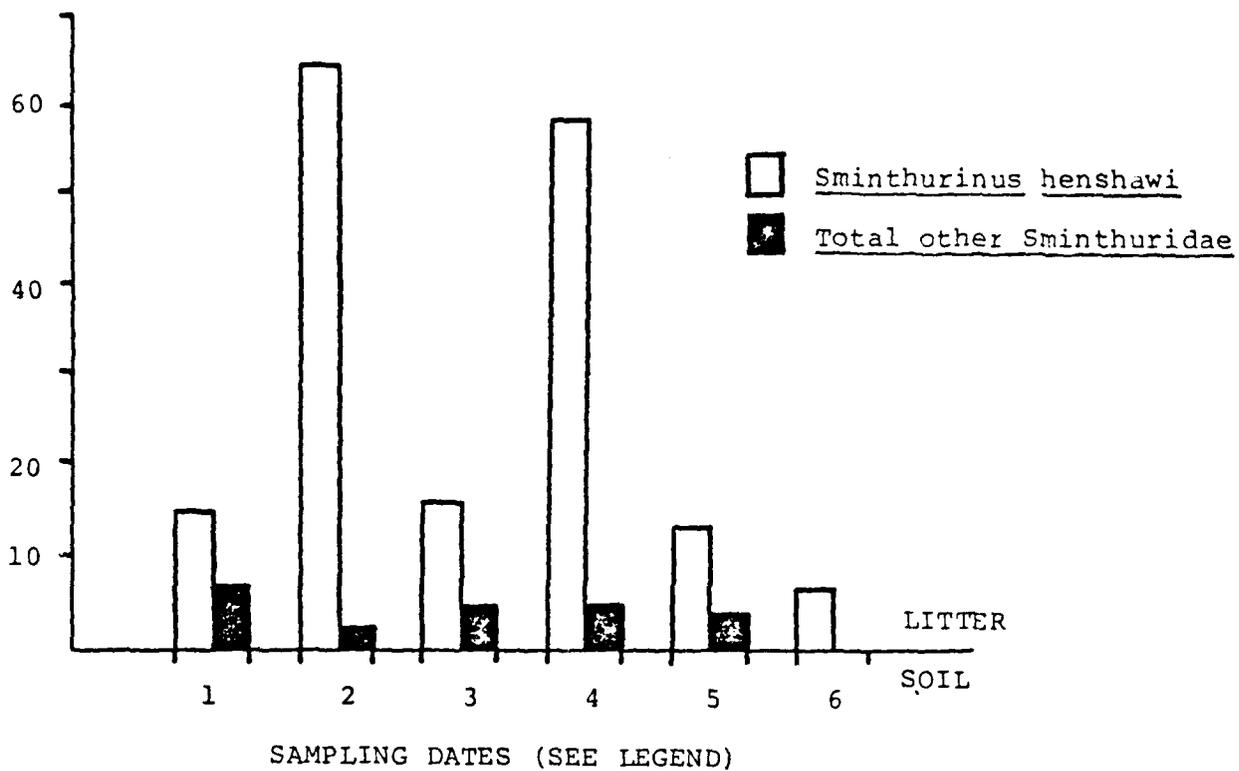
Key to dates (1 through 6):

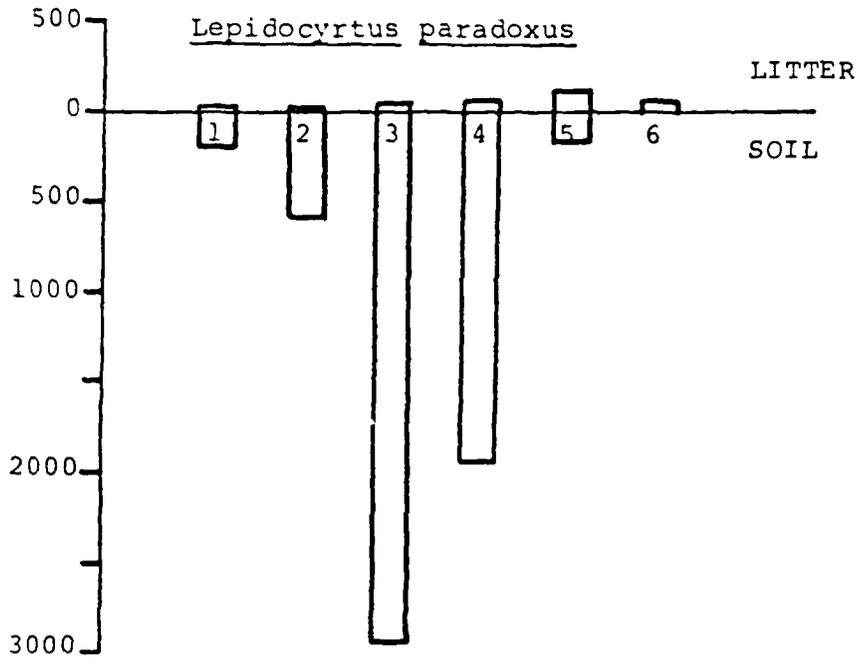
- 1 - August 10-12 (litter), August 16 (soil);
- 2 - August 25-27 (litter), August 30 (soil);
- 3 - September 7-9 (litter), September 14 (soil);
- 4 - September 22 (litter), September 28 (soil);
- 5 - October 4 (litter), October 11 (soil);
- 6 - October 22 (litter only).

For reasons of clarity, soil and litter data from samples taken 4 to 6 days apart are lumped as one date in Figs. 42-52.

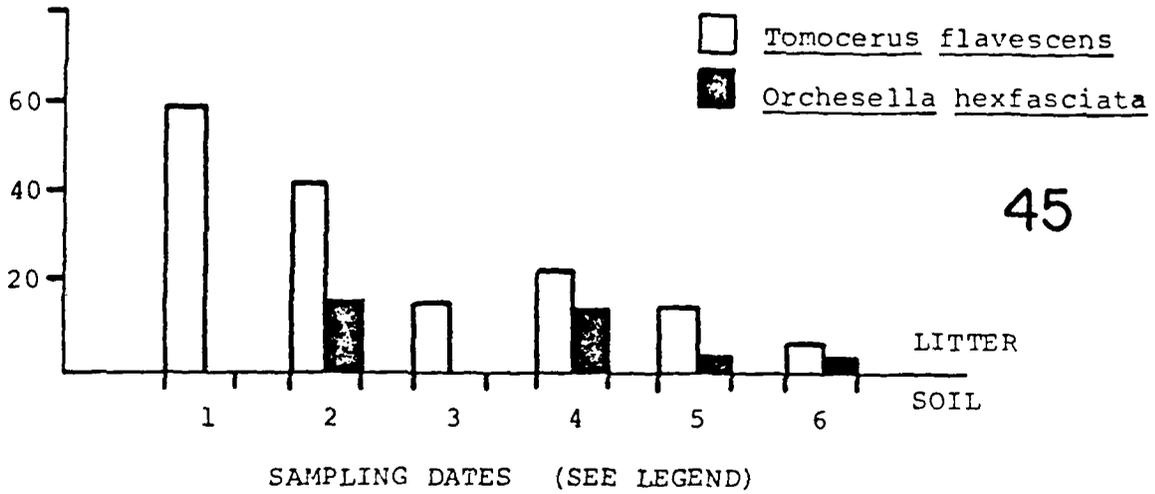


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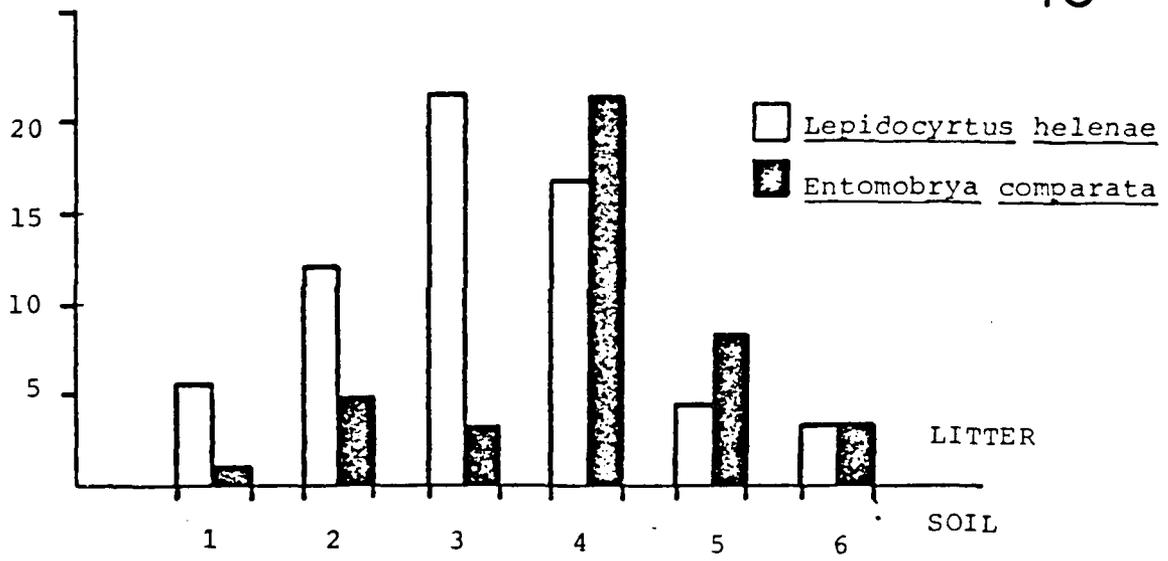


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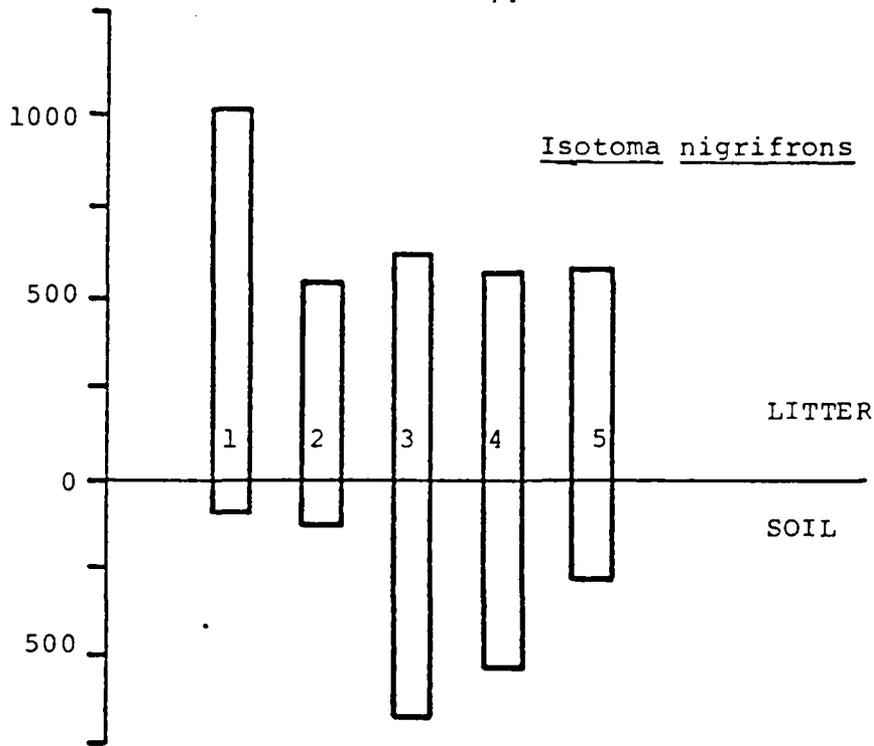


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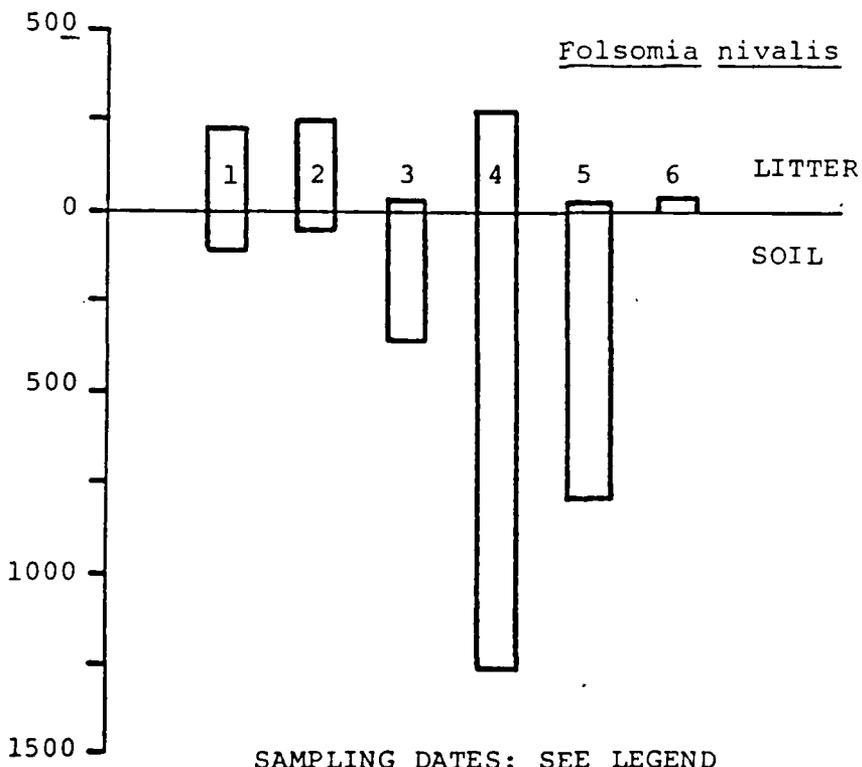
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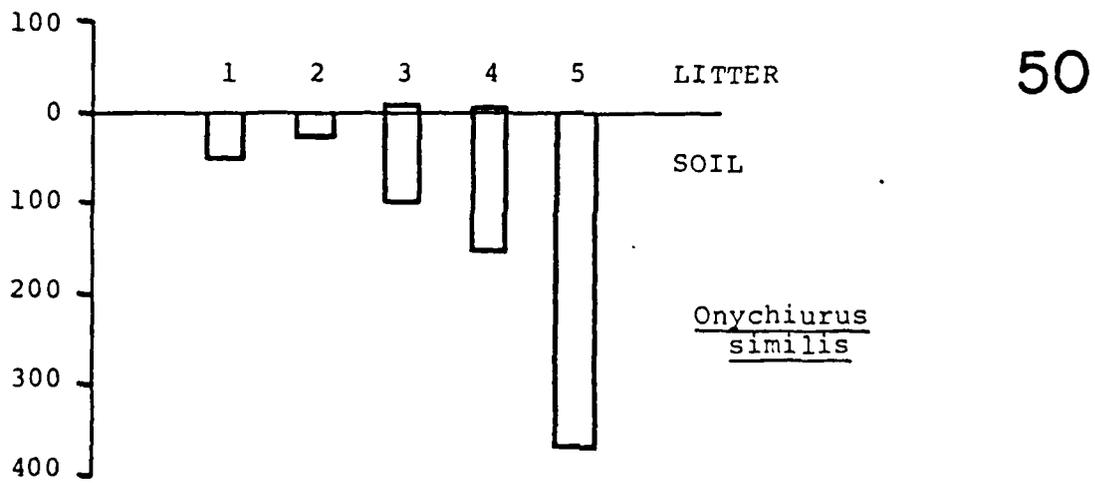
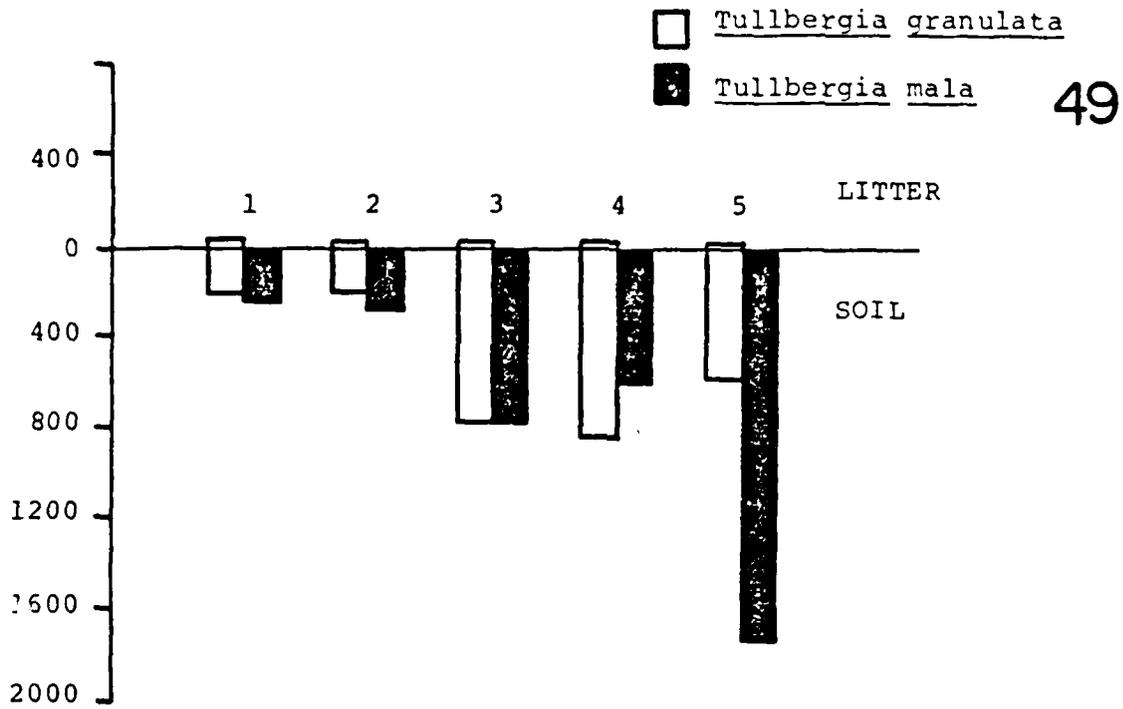
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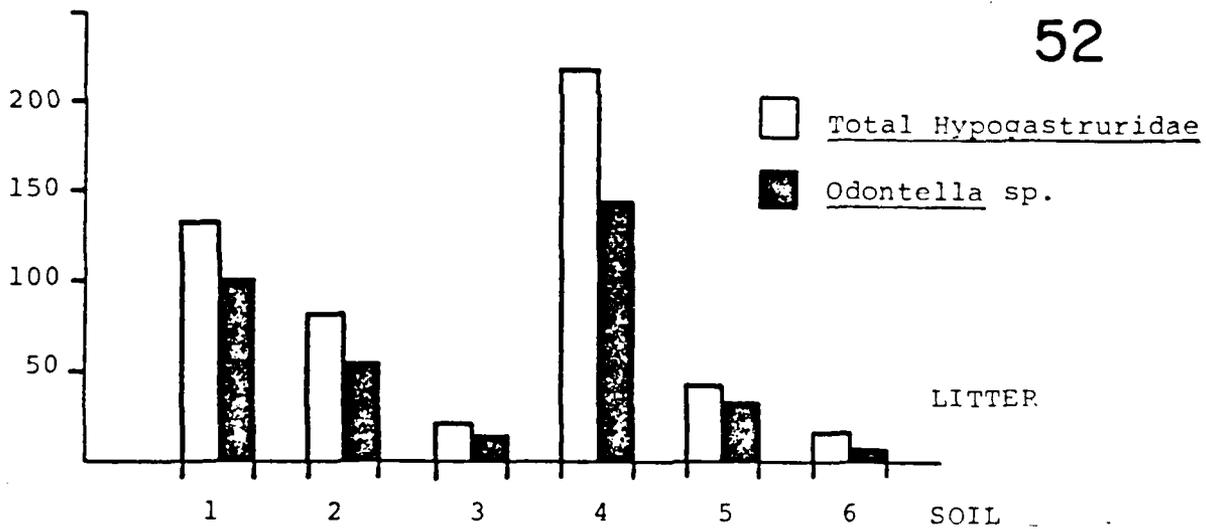
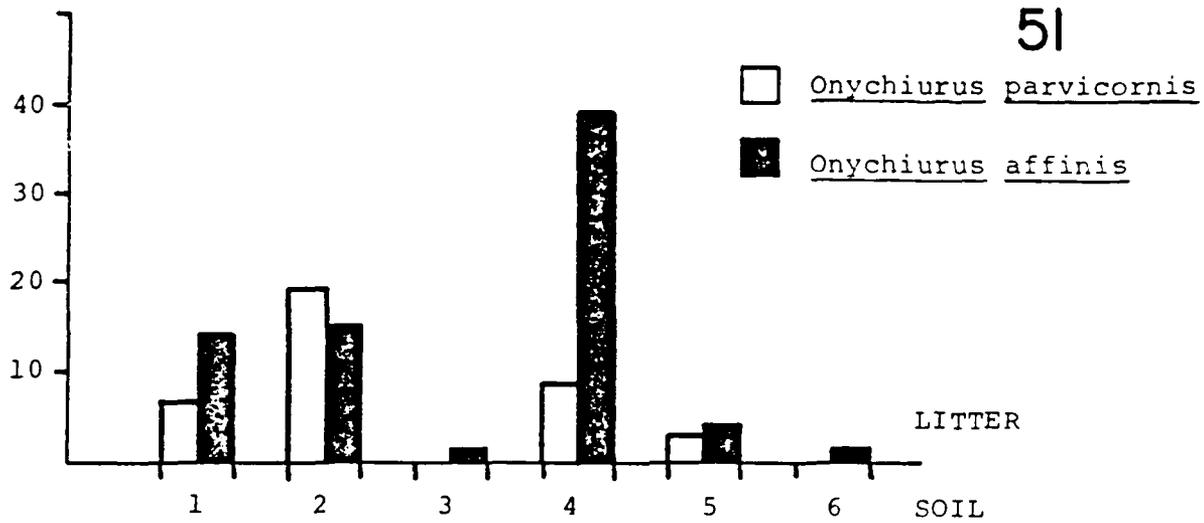
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SAMPLING DATES (1 - 5): SEE LEGEND



SAMPLING DATES (SEE LEGEND)

similar apparent paradoxes regarding occurrence of soil forms high in the litter-soil profile (Hagvar 1983; Haarlov 1960).

The majority of Hypogastruridae (Table 12) do not occur in soil at all. A species of Odontella dominates the group (70%); its population fluctuations, i.e. two peaks between August and October, parallel the trend shown by all hypogastrurids combined (Fig. 52). The family exemplifies the general tendency of litter-inhabitants to fluctuate more widely, and at shorter intervals, than euedaphic populations.

b. Acari:

Families comprising the mite community are listed in Table 13. Peak density estimates are given for those dates on which sample frequency of a given family was $\geq 20\%$.

Several groups, particularly of Prostigmata, occupy both litter and soil habitats. Preference for litter strata is strongly indicated for families which occur with an overall frequency $> 20\%$, i.e. Uropodidae, Zerconidae, and the prostigmatid Pyemotidae (Table 13). Pyemotids are probably entirely predaceous (Krczal 1959), while uropodids are mostly fungivores (Athias-Binche 1977). The latter author also observed, in forest floor uropodids, a definite reluctance to leave the litter layer, except under conditions of extreme drought.

Oribatids (Fig. 53) and Eupodidae (Fig. 54) show parallel fluctuations both in numbers and vertical distribution. Large populations in late September are almost equally distributed between litter and soil. In October, however, the litter layers are largely abandoned and soil now harbors 80% or more of the populations.

Standard errors of population means are generally large

(Table 13), indicating strong aggregation tendencies. Various indices may be used to quantify these tendencies (e.g. Southwood 1978, critical comparison by Cancela da Fonseca and Stamou 1982). Preliminary tests of common mite groups at Turner Rd., (Chi-square against Poisson) only confirm the obvious. However, it has been shown that aggregation tendencies can vary significantly with season (Athias-Binche 1977; Geoffroy et al 1981; Cancela de Fonseca and Stamou 1982) and we prefer to wait for further (1983) data for a fuller description of acarine dynamics.

Overall, mite densities tended to decline sharply in mid-August, followed by a September-October peak. Seasonality is most strongly apparent in Oribatida (Fig. 53) and prostigmatids (Figs. 54 to 58). This observation is supported by others (e.g. Evans et al 1961), conclusions regarding Mesostigmata being hindered by their generally low frequency in samples (for this reason, estimates of densities in soil are added only for selected dates in Figs. 58-61).

Judging by a number of reports on acarine faunas in north temperate regions (reviewed by Wallwork 1970), a spring or summer peak may also have occurred, and appears in our 1982 data as relatively high abundances in early August. The distinctness of these fluctuations depends on the reproductive cycle of a given taxon and may be strongly influenced by the dominant species within that taxon (Lebrun 1964, 1965; Block 1965; Garay 1981).

c. Diplopoda:

Three species of millipedes (Table 14), each belonging to a different order (Polyzoniida, Julida, Chordeumatida, following Hoffman 1979) are found at Turner Rd. They also occur in all sites sampled or collected so far, and seem to be generally common in deciduous forests in the ELF area.

Table 13. Peak densities/m² of Acari in litter and soil, Turner Rd 1982; + present in low numbers; - never present in samples; D% indicates dominance relative to other families in the same suborder; (F%) = frequency over all samples (n=100 for soil samples, n=120 for litter samples).

		(Month) Density/m ² + SE (F%)	
	D%	SOIL	LITTER
Oribatida		(Sep.) 3525+836 (79.0)	(Sep.) 2262+371 (95.0)
Mesostigmata			
Uropodidae	13.1	-	(Sep.) 131.2 +50.9 (25.8)
Rhodacaridae	1.9	+ (6.0)	(Sep.) 15.2 + 7.2 (7.5)
Parasitidae	13.2	(Sep.) 200+105 (17.0)	(Oct.) 44.8 +10.9 (61.7)
Veigaiidae	8.4	(Sep.) 325+155 (18.0)	(Aug.) 42.4 +20.3 (43.3)
Macrochelidae	1.	-	(Sep.) 11.2 + 4.3 (15.0)
Ascidae	14.1	(Sep.) 275+135 (12.0)	(Aug.) 100 +89.3 (29.2)
Phytoseiidae	2.1	+ (2.0)	(Sep.) 14.4 + 5.6 (15.8)
Digamasellidae	3.2	+ (4.0)	(Oct.) 14.2 + 8.5 (11.7)
Zerconidae	7.9	-	(Aug.) 65.6 +41.8 (22.5)
Ologamasidae	6.7	+ (6.0)	(Sep.) 66.4 +18.9 (25.0)
Epicriidae	0.8	-	(Sep.) 8 + 3.8 (8.3)
Ameroseiidae	0.5	-	+ (7.5)
Parholaspididae	0.1	-	+ (1.7)
Sejidae	0.1	-	+ (0.8)
Prostigmata			
Eupodidae	41.6	(Oct.) 1275+335 (53.0)	(Sep.) 1552.8+432.5 (90.0)
Tydeidae	19.7	(Oct.) 300+205 (15.0)	(Oct.) 616 +173.4 (72.5)
Rhagidiidae	4.9	(Oct.) 1250+219 (59.0)	(Sep.) 103.2+21.3 (59.2)
Pygmephoridae	16.5	(Oct.) 1175+930 (13.0)	(Sep.) 492 +195.8 (54.2)
Scutacaridae	1.8	(Oct.) 200+110 (8.0)	(Sep.) 51.2+25.9 (25.9)
Alicorhagiidae	6.5	(Oct.) 250+150 (8.0)	(Aug.) 290.4+74.2 (28.3)
Pyenotidae	3.0	-	(Aug.) 104 +72.8 (21.7)
Blellidae	2.3	+ (7.0)	(Aug.) 59.2+19.0 (57.5)
Erythraeidae	0.5	+ (3.0)	(Aug.) 12.8+ 5.8 (30.0)
Tarsonemidae	1.3	+ (1.0)	(Sep.) 47.2+ 6.1 (29.2)
Nanorchestidae	0.9	+ (1.0)	(Sep.) 46.4+33.6 (20.0)
Ereynetidae	0.6	-	(Sep.) 13.6+ 8.5 (18.3)
Johnstonianidae	0.1	-	+ (5.8)
Pachygnathidae	0.3	+ (2.0)	+ (10.8)
Cunaxidae	0.1	-	+ (3.3)
Adamystidae	0.01	-	+ (0.8)
Cheyletidae	0.02	-	+ (0.8)
Tetranychidae	0.01	-	+ (0.8)
Alcyidae	0.01	-	+ (0.8)
Prostigamata			
Penthaleidae	0.02	+ (1.0)	-
Astigmata			
Acaridae	88.9	+ (2.0)	(Sep.) 31.2+17.1 (10.8)
Glycyphagidae	7.9	+ (3.0)	+ (0.8)
Histiostomatidae	3.2	-	+ (0.8)

Figs. 53 - 61. Density/m² ± one SE of Oribatida and common mite families in litter and soil (n = 20 samples), Turner Rd 1982. Densities in soil ± SE (for Mesostigmata) are added for single dates on which frequency of occurrence was at least 20%. Key to dates (1 through 6) as in preceding Figures.

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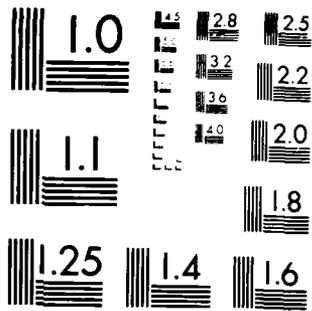
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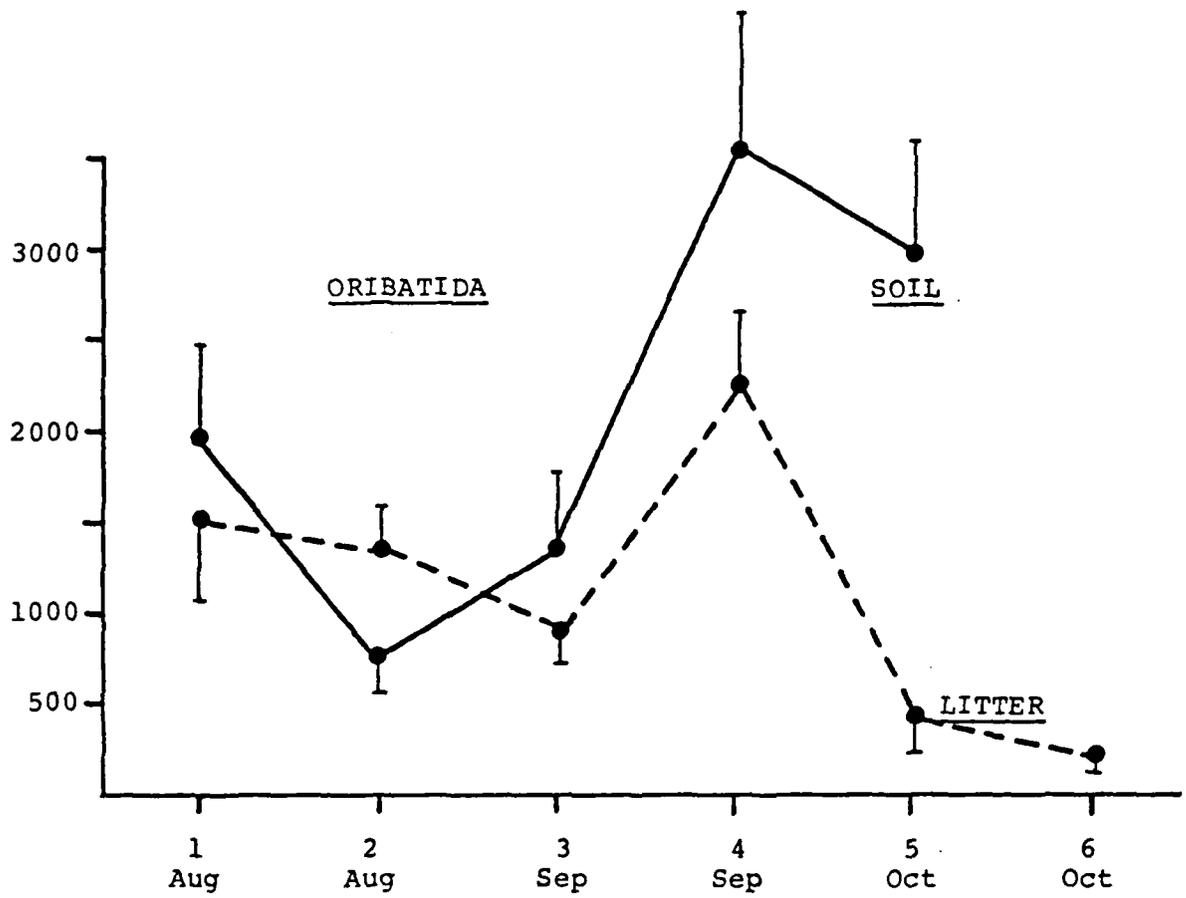
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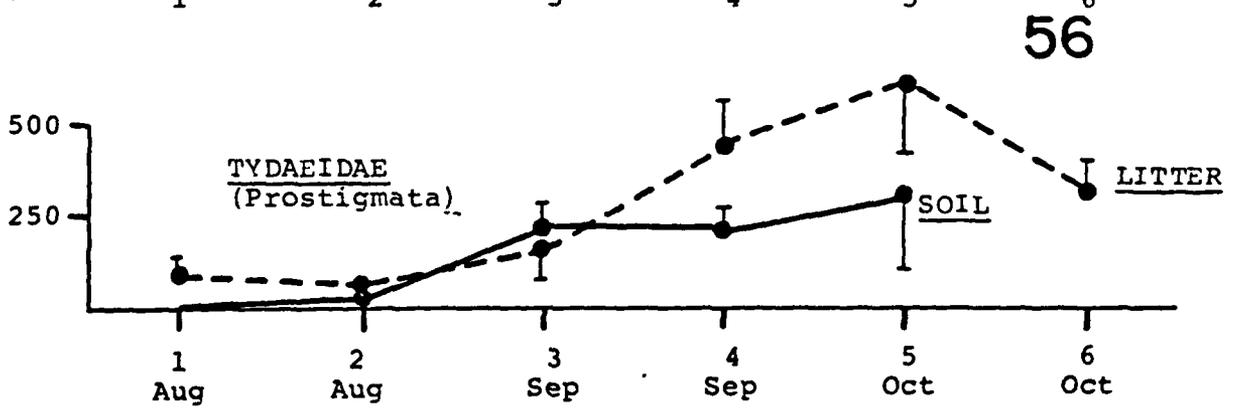
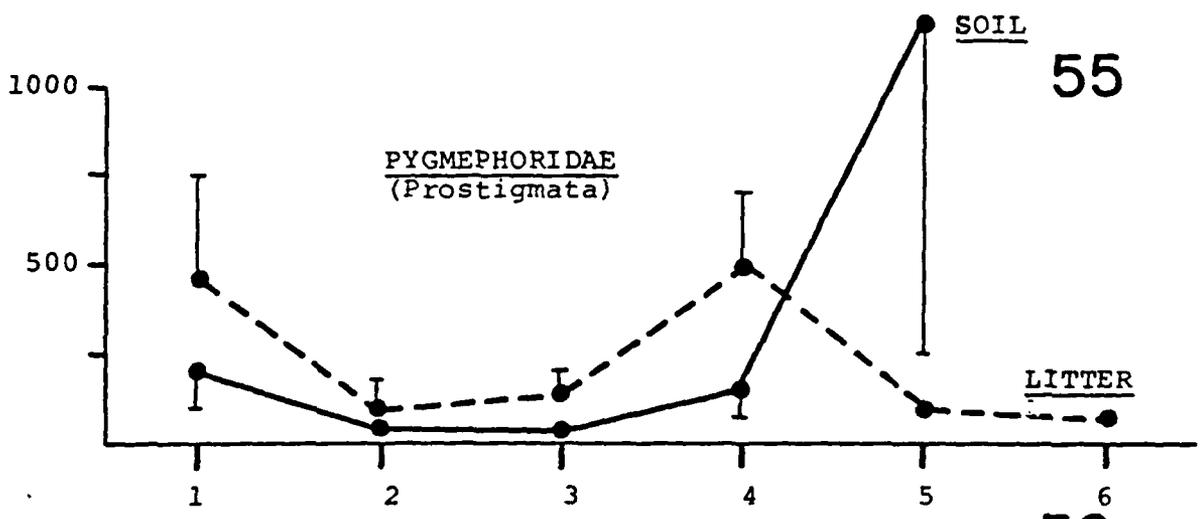
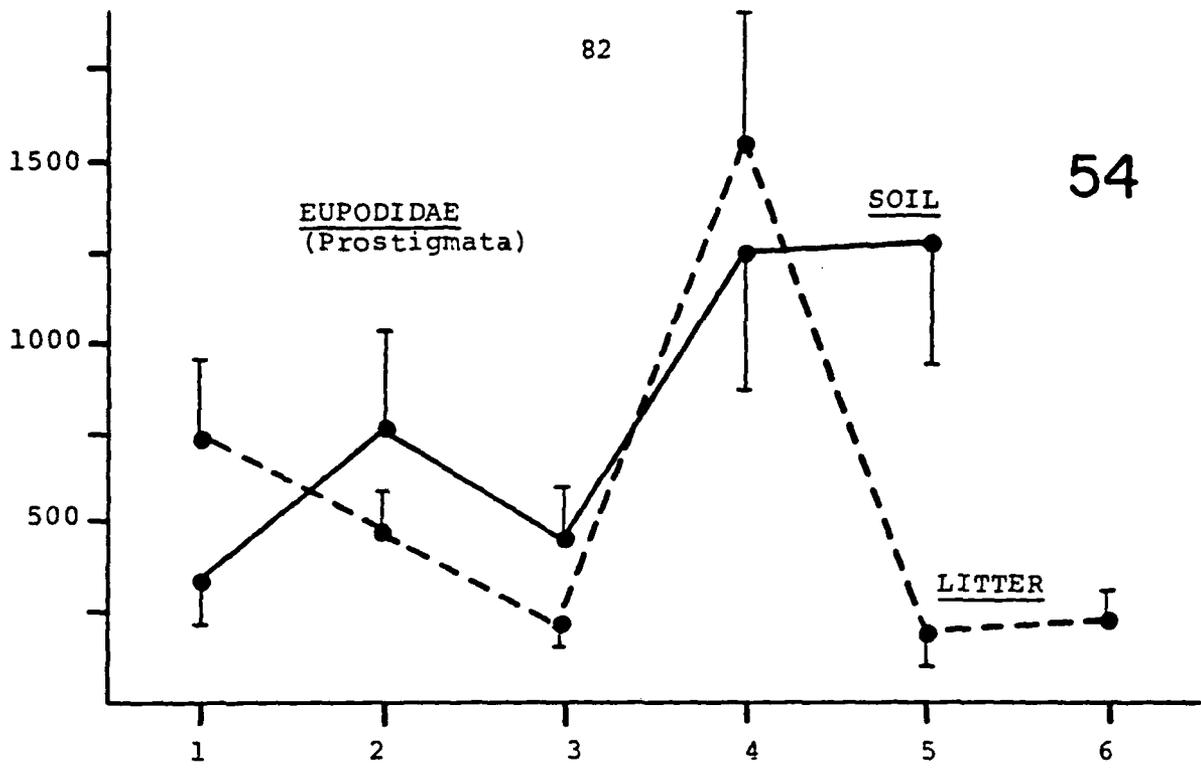
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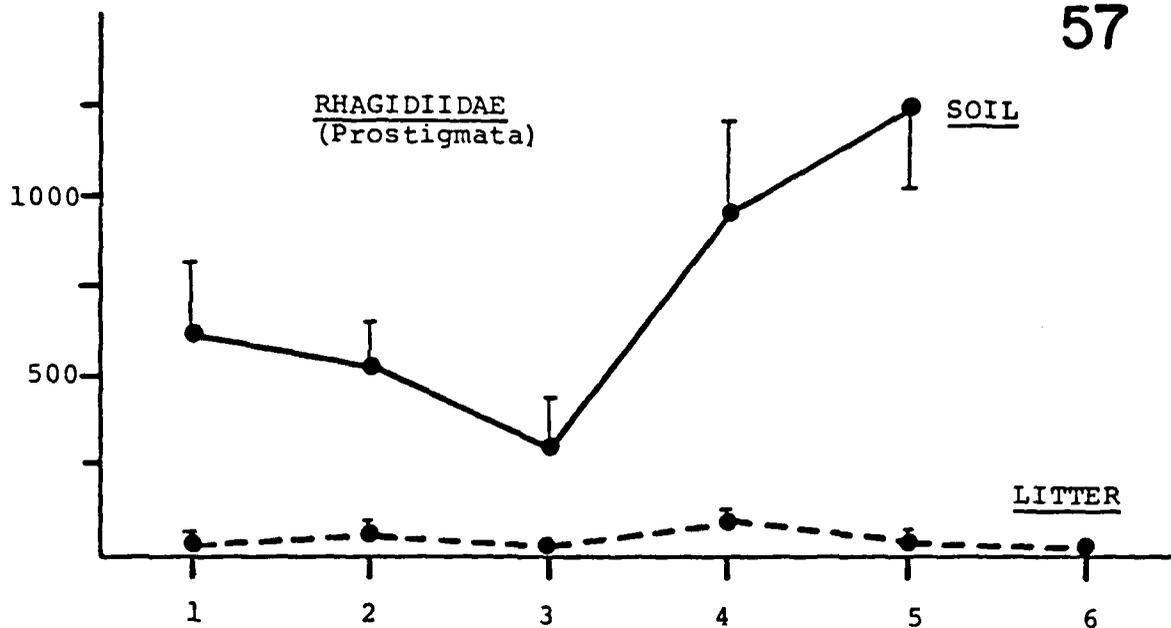


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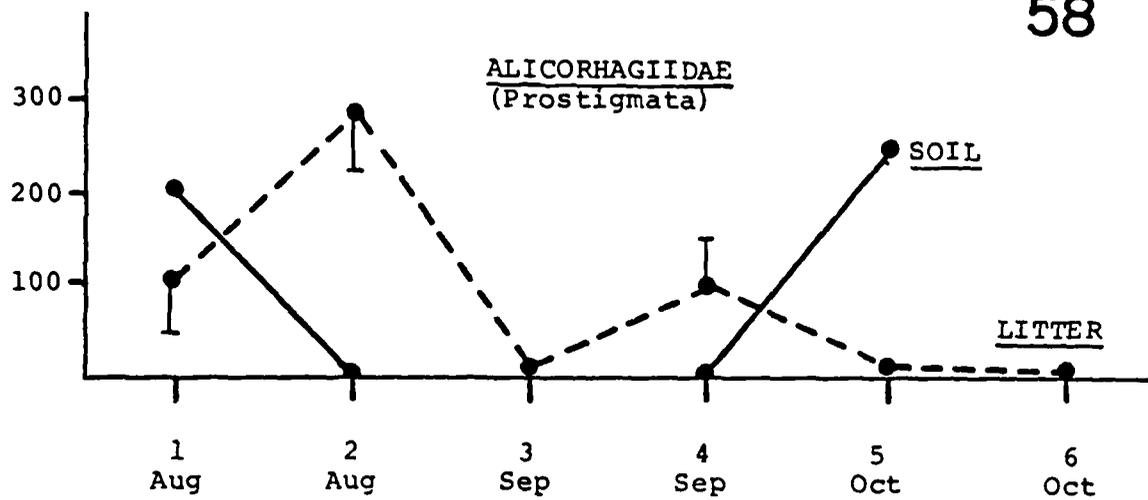


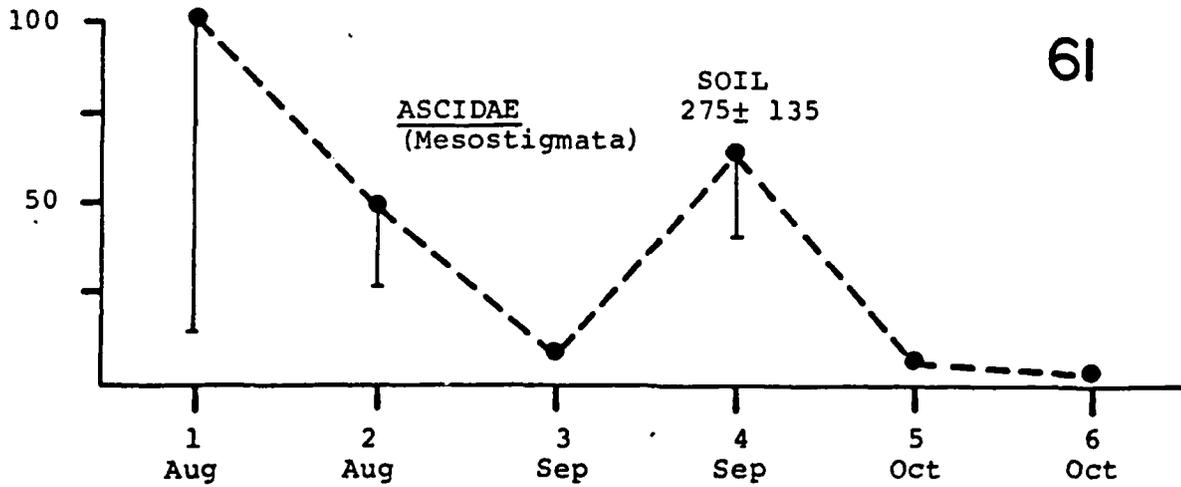
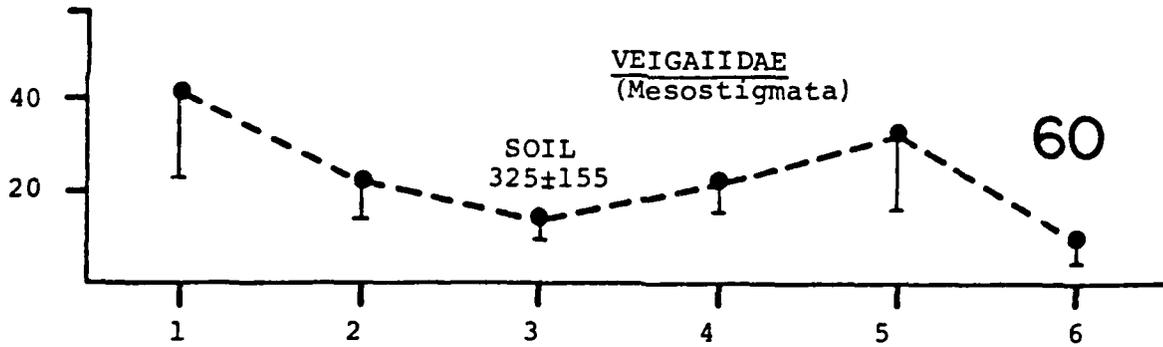
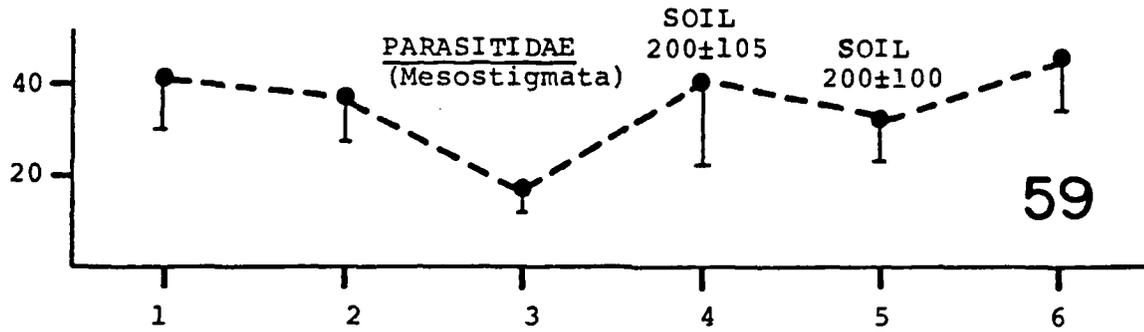
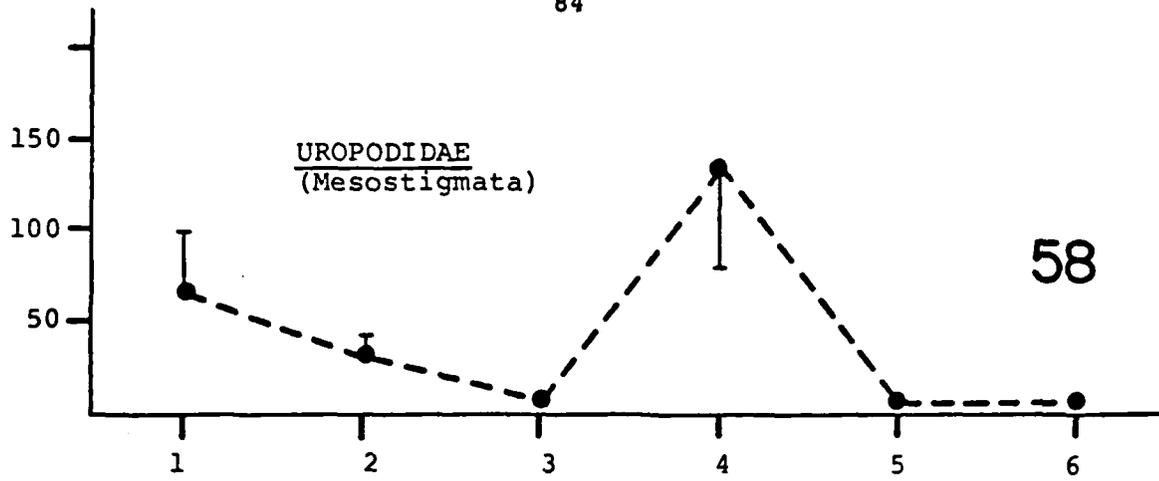


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Frequency of all species was well below 10% in soil samples. It is probable that they spend some time in the soil (Johnson 1952), especially in immature stages, which are sometimes recovered while handsorting soil in search of other taxa.

Table 14. The Diplopoda at Turner Rd.: peak densities in litter and overall dominance values by species.

Family/species	(D%)	density/m ² + SE (month)	
		SOIL	LITTER
Cleidogonidae			
<u>Cleidogona exaspera</u>	(57)	+	15.5 ± 2.3 (Sep.)
Parajulidae			
<u>Uroblaniulus canadensis</u>	(32)	+	11.2 ± 3.3 (Sep.)
Polyzonidae			
<u>Polyzonium bivirgatum</u>	(11)	-	+

Overall, Cleidogona exaspera is dominant (57%), followed by Uroblaniulus canadensis (Table 14). In litter, density estimates for all species combined show a September peak comprised mostly of C. exaspera and U. canadensis.

Average densities of millipedes in deciduous forests generally lie between 20 and 100 ind/m² (e.g. Geoffroy 1981; Ghilarov 1979, Lebrun 1971, van der Drift 1951), so that Turner Rd values appear quite low. They are, however, underestimates with respect to the site as a whole: based on hand-collecting experience, rotting wood and subcortical habitats harbor relatively large numbers of diplopods, U. canadensis in particular.

Considering litter/soil habitats alone, diplopods are a relatively unimportant segment of the site's macrodecomposers, this trophic group being dominated by lumbricids (see Section F).

d. Predatory macroarthropods

Centipedes, spiders, opilionids and certain families of Coleoptera can be grouped, based on feeding habits and body size (Swift et al 1979), as macropredators. We include pseudoscorpions in this category, somewhat marginally, in order to effect a clearer distinction between predatory mites and predatory macroarthropods.

Virtually all spiders (Table 15) were extracted from litter, not soil. Because most of them were immature and could not be identified beyond family level, dominance indices in Table 15 are based on total numbers/family over all dates. Species densities are included only for adults occurring with frequency $\geq 20\%$ on the date of their peak abundance; none of these adults showed clear seasonality trends.

Table 15. Peak densities \pm SE and family dominance indices, for spiders extracted from litter, Turner Rd. 1982.

Family/species	D%	density/m ² SE (month)	
		LITTER	
Amaurobiidae	33.9	12.8	\pm 5.6 (Sep.)
<u>Callioplus borealis</u>		4.8	\pm 2.1 (Aug.)
Linyphiidae	24.4	10.4	\pm 3.5 (Sep.)
<u>Centromerus persoluta</u>		4.8	\pm 1.6 (Sep.)
Micryphantidae	17.3	8.0	\pm 4.9 (Aug.)
Theridiidae	10.7	7.2	\pm 3.4 (Sep.)
<u>Ctenium riparius</u>		3.2	\pm 1.7 (Sep.)
Lycosidae	5.4	4.0	\pm 1.9 (Aug.)
<u>Pirata marxi</u>		3.2	\pm 1.7 (Sep.)
Thomisidae	4.8		+
Salticidae	1.2		+
Agelenidae	1.2		+
Hahniidae	0.6		+
Clubionidae	0.6		+

Spiders as a whole were most abundant in late September (Fig. 32); Amaurobiidae, Linyphiidae and Micryphantidae dominated the spider association at Turner Rd on all dates.

Chilopoda (Table 16) were dominated by geophilids, of which S. chionophila was most abundant both in litter and soil. Johnson (1952) maintained that the species preferred the litter habitat and only retreated into humus under dry conditions. We have evidence to the contrary: in 1983, while handsorting humus and soil samples for

Table 16. Peak densities and dominance of Chilopoda in litter and soil, Turner Rd 1982.

Species	D%	density/m ² SE (month)	
		SOIL	LITTER
Geophilomorpha			
<u>Strigamia chionophila</u>	60.7	175 + 85 (Oct.)	35.2 + 7.7 (Sep.)
<u>S. branneri</u>	28.4	-	12.8 + 4.0 (Sep.)
<u>Taiyuna opita</u>	9.0	+	7.2 + 2.7 (Aug.)
Lithobiomorpha			
<u>Nadabius iowensis</u>	1.9	+	+

Table 17. Densities of geophilomorph chilopods in humus and soil (1/16 m², depth 25-35 cm) of Test and Control sites; n=20/site/date.

Date, 1983	density/m ² + SE		
	TEST	CONTROL	TURNER RD.
Aug. 8	22.1 + 1.8	43.2 + 11.0	-
Aug. 22	15.2 + 4.8	67.2 + 11.5	-
Sept. 26	-	-	101.6 + 13.9
Oct. 3-10	30.4 + 8.0	127.2 + 20.5	-
Oct. 17	-	-	56.8 + 6.7

Dominant species:

TEST: Taiyuna opita 62%

CONTROL: Strigamia chionophila 79%

TURNER RD: S. chionophila 61%

earthworms, any centipedes found were also collected. In general, humus subsamples contained the vast majority of individuals, but some were also found at 0-10 and 10-20 cm depths. A preliminary summary (Table 17) indicates high chilopod densities in soil, much exceeding those in litter (Table 16). With the exception of Roberts' (1956, cited in Lewis 1981) density estimates of 400 to 900/m², geophilid populations at Turner Rd. can be considered abundant in comparison to other available estimates (Lewis 1981).

Pseudoscorpions, of negligible importance in soil, reached densities of 40 individuals/m² in litter (Fig. 34). We found two species, Microbisium confusum and Mundochthonius rossi, in equal numbers and approximately equal frequencies, and almost all immature. We intend to describe the autecology of these species once sufficient material has accumulated. M. confusum is reported to be parthenogenetic, although males have been found in Michigan populations (Nelson 1975).

Summary data on Coleoptera are given in Table 18. Densities in soil are questionable because of low sample frequencies even at "peak". Litter harbored a number of beetle families not shown here, notably Curculionidae and Cryptophagidae.

Table 18. Peak densities of total Coleoptera, Carabidae and Staphylinidae in litter, 1982.

	No./m ² SE (month)	
	SOIL	LITTER
Coleoptera total ?	125 \pm 50 (Aug.)	53.6 \pm 9.4 (Sep.)
Carabidae	-	4.8 \pm 1.9 (Sep.)
Staphylinidae ?	75 \pm 41 (Aug.)	23.2 \pm 6.8 (Sep.)

Of the major predators, carabids were extracted very

infrequently (a September density of $4.8/m^2$ is derived from samples with a carabid frequency of 25%). Densities of single species in deciduous forests range from <1 to $3.7/m^2$ (Szyszko 1976; Carter and Cragg 1976; Carter 1980), based on studies using random quadrat sampling (most authors employ pitfall traps, which do not yield density values). Our estimate of $4.8/m^2$ for late-season densities of carabids may thus be fairly accurate.

Staphylinidae were more frequent and more abundant than any other family in litter samples (dominance value for 1982 = 63%). Highest density, $23/m^2$, was recorded in September, lowest on October 22 ($10/m^2$); seasonal fluctuation was relatively minor.

With the data we have available for fall of 1982, preliminary dominance relationships between various macropredator groups can be estimated (Table 19). Potential and known sources of bias are as follows:

- Larval Coleoptera are excluded because they were not further identified and the proportion of predaceous forms among them is not known;

- Opiliones may in fact be more abundant than Table 19 indicates: their initial inclusion in, and extraction from, $1/16m^2$ litter samples is of questionable accuracy, although sample replication (20) should have been more than enough to assess density of opilionids (Dudich et al 1952; Reise and Weidemann 1975);

- Staphylinidae are included, based on the assumption that the great majority of them are predaceous (Borror et al 1976), although an unknown proportion of them may have been non-carnivorous.

Based on total numbers obtained August through October

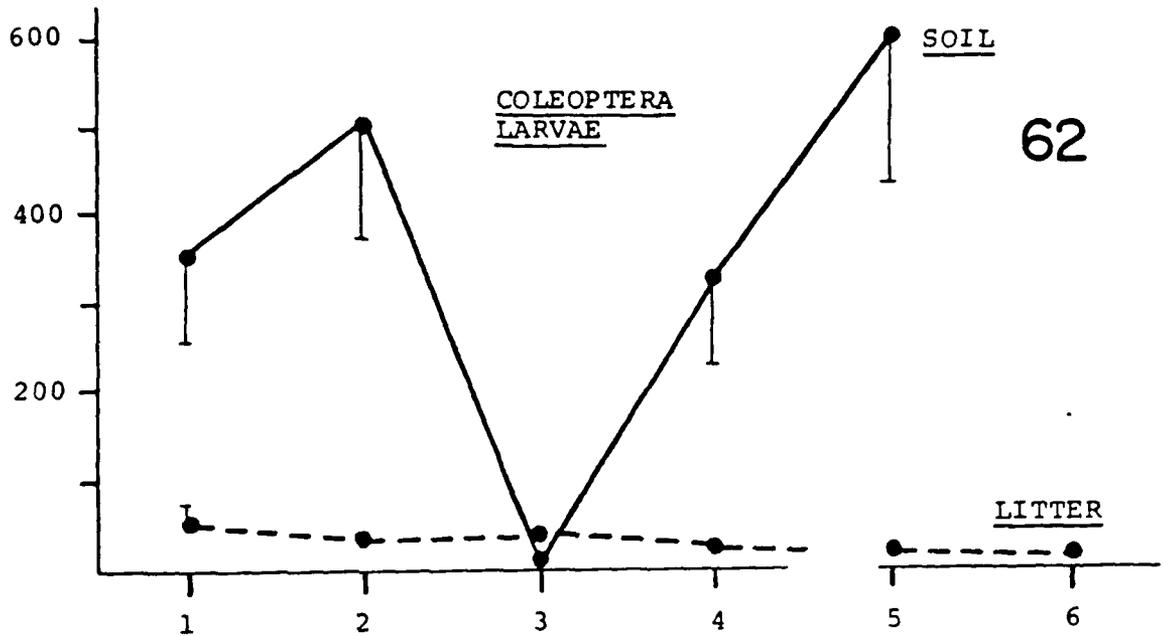
(Table 19), litter-inhabiting predators were dominated by spiders (40%), followed by chilopods (25%), pseudoscorpions (18%) and staphylinids (14%). In terms of numbers as well as biomass, spiders usually dominate forest floor predator associations (Geoffroy et al 1981; Huhta 1965; Albert 1979). The subdominant status of chilopods (almost exclusively geophilids) is unusual (Albert 1979) and seems to be characteristic of the Turner Rd species assemblage. Judging by preliminary Test/Control data (e.g. Table 17), it may be equally characteristic of the arthropod community in definitive sites.

Table 19. Litter-inhabiting macropredators: total numbers (August through October 1982) and % dominance.

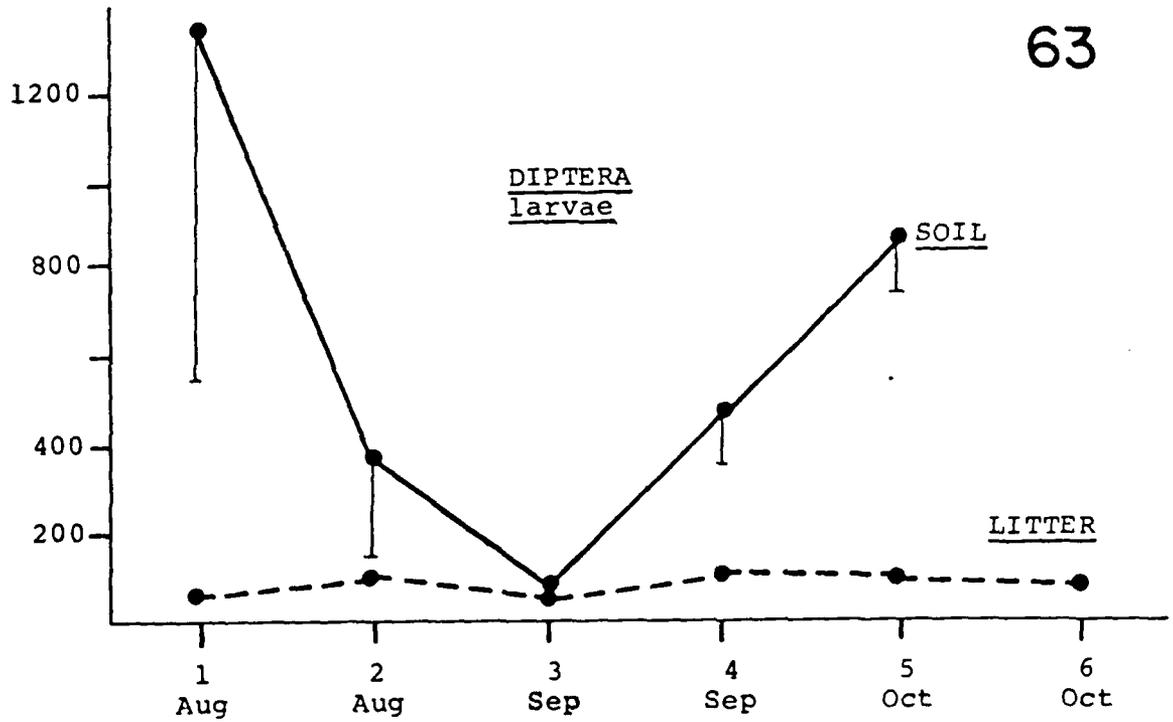
Taxon	N	D%
Aranei	337	40.1
Chilopoda	211	25.1
Pseudoscorpiones	151	18.0
Staphylinidae	120	14.3
Carabidae	17	2.0
Opiliones	4	0.5

e. Insect larvae and minor taxa:

Larval insects constituted a numerically important segment of Turner Rd forest floor arthropods. Abundance of dipteran and coleopteran larvae as illustrated in Figs. 62 and 63) are likely to be underestimates because techniques other than heat extraction are more suitable for them (Healey and Russel-Smith 1970). However, the relative prevalence of Diptera (Fig. 63) was quite obvious, and reiterates similar density relations reported for other forest ecosystems (Geoffroy et al 1981; Altmüller 1976; Schauer mann 1976; Bornebusch 1930).



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Figs. 62 - 63. Density \pm one SE of larval Coleoptera and Diptera in litter and soil, Turner Rd, 1982 (for exact dates, 1 through 6, see legends to preceding graphs).

A distinct decline in numbers of soil-inhabiting fly and beetle larvae (Fig. 62 and 63) on September 14 could not be correlated to moisture conditions (see precipitation data in Fig. 41). It is likely that dominant groups had matured prior to the sampling day, and that autumnal reproduction later replenished the soil-dwelling larval populations.

Schauer mann (1979) gives detailed analyses of dipteran population dynamics in forest soil and litter in Germany, at a site dominated by members of the family Sciaridae, Cecidomyidae being sub-dominant. We have evidence, although highly circumstantial, that Turner Rd dipteran populations in August were dominated by Cecidomyidae: heat-extraction of soil samples (an inappropriate technique!) produced large numbers of cecidomyid adults from each sample. Densities estimated from these data were 5400/m² (Aug. 16), 1225/m² (Aug. 30), and 100/m² (Sept. 14). Compared to other Diptera, August dominance values for cecidomyids reached 95%. With caution, we may thus interpret the decline in larval abundance (Fig. 63) as caused by a wave of maturation of cecidomyids, which were able to complete development during the 4-6 day extraction time.

Sciaridae, on the other hand, showed peak emergence in late September - early October, mostly from litter samples. In spite of the inappropriateness of the technique, soil and litter data validate each other, with respect to maturation time, for both families. Whether the mid-summer peak of cecidomyids indicates that the family is univoltine, as some forest soil Diptera seem to be (Schauer mann 1979) will have to be determined through 1983 data.

The order Psocoptera was represented mostly by wingless forms,

especially in the family Pseudocaeciliidae which preferentially inhabited soil (Table 20). Epipsocidae and Polypsocidae, absent from soil, were dominated by the former family. In general, frequencies of Psocoptera in litter samples (65% maximum) was higher than in soil samples (30% maximum).

Protura (Eosentomon pallidum) (Table 20) were also extracted infrequently (25% maximum). Little information is available on this order, the members of which are constant, but low-density inhabitants of forest soils (Tuxen 1949).

Table 20. Peak densities of Protura and Psocoptera, 1982, in litter and soil.

TAXON	no./m ² + SE (month)	
	SOIL	LITTER
Psocoptera		
Epipsocidae	-	11.2 ± 4.0 (Aug.)
Polypsocidae	-	+
Pseudocaeciliidae	150 ± 65 (Sep.)	+
Protura		
<u>Eosentomon pallidum</u>	175 ± 75 (Sep.)	+

E. Surface-active arthropods

1. Introduction:

Information on surface-activity of arthropods at Turner Rd. is as yet available only for the 1982 half-season, except for carabid Coleoptera and Opiliones, all 1983 samples of which have been identified. We will present data on most taxa in abbreviated form, since late-season trapping provides a very limited idea of a species' activity patterns. Carabid activity, however, will be discussed in greater detail.

2. Methods:

Traps: in 1982, we used clear plastic cups (8.5 cm rim diameter). They were replaced by white plastic cups with a snap-in funnel insert (rim diameter 9.0 cm) in 1983. Ethylene glycol was the trapping medium; upon collection, catches were rinsed with and stored in 95% ethanol. Traps were distributed randomly throughout the site, in permanent emplacements. Distance between any two traps was ≥ 10 m.

Schedules and replication: the main portion of Turner Rd was used for diel trapping: at intervals of 7 days, traps were activated at dusk, emptied and re-activated at dawn, and collected again at dusk (consecutive night/day periods). Replication 1982: 12 diel traps (8 dates); 1983: 20 traps (25 dates).

Faunal material: certain winged insects, i.e. Diptera, Hymenoptera and Lepidoptera, ants, and taxa trapped in low numbers, are not included in the discussion of results.

Climate data: no on-site temperature and precipitation records are available for 1982-83. Where appropriate, we will make use of records obtained from a NOAA weather station at Crystal Falls, approximately 24 km from Turner Rd.

3. Results and Discussion

3.1. The common surface-active taxa:

August through September catches in 1982 were not numerically large, with a few exceptions. From day/night totals given in Table 21, a few generalizations can be derived; hopefully they will be tested once 1983 data are complete. Carabidae as a group were mostly night-active, while staphylinids and leiodids were predominantly day-active. Twenty additional beetle families captured in traps were each represented by only a few individuals. Opiliones, too, were generally night-active, as were Collembola and Orthoptera (Table 21).

Table 21. Total diel catches of arthropods in pit-traps at Turner Rd. 1982: 8 dates Aug. 4-Sept. 22; 1983: 25 dates May 5-Oct. 27.

TAXON	TOTAL NO.	NIGHT	DAY
<u>1982</u> Coleoptera:			
Carabidae	103	73	30
Staphylinidae	32	6	26
Leiodidae	84	10	74
Opiliones	18	17	1
Aranei	57	19	38
Arcari total	196	87	52
Hypopi (Astigmata)	57	44	13
Collembola	213	140	73
Orthoptera	12	11	1
Psocoptera	24	13	11
<u>1983</u> Coleoptera:			
Carabidae	663	515	148
Staphylinidae	285	92	193
Leiodidae	162	38	124
Opiliones	107	78	29

Further breakdown to family or species level allows the following conclusions regarding the most frequently caught taxa:

Collembola: Species exhibiting the highest density within hemeidaphic families (ref. to Table 12) were most commonly caught in traps. Of 69 individuals of S. henshawi and S. lepus, 66% were trapped during the day. The entomobryid T. flavescens, as well as the dominant isotomid I. nigrifrons were clearly night-active (85 and 92%, respectively, caught in nocturnal traps). Due to low numbers/date, seasonality patterns did not emerge clearly for any Collembola species.

Aranei: Micryphantidae, Linyphiidae and Lycosidae all tended to be diurnal; no further conclusions can be drawn at this time.

Acari: Eupodidae, Tydaeidae and Erythraeidae made up the bulk of trapped Prostigmata, Phytoseiidae and Parasitidae were virtually the only Mesostigmata ever caught. Hypopi (phoretic deutonymphs of Astigmata) are included in Table 21 in order to confirm observations made at the Silver Lake site: most numerous in August, they virtually disappear thereafter, although taxa observed to be their phoretic hosts (spiders, beetles, opilionids) are still relatively frequent.

Opiliones: A total of five species were trapped over both seasons, two of them with fair frequency (Leiobunum nigripes and Odiellus pictus). Caddo boopis (1 individual) and Sabacon crassipalpe (8 individuals, all in September) were caught in 1982, but were never trapped thereafter. Leiobunum politum was equally rare (5 individuals, July-August 1983).

Monthly totals, divided into night and day occurrences, are

illustrated in Fig. 64 and 65 for the two dominant species. What little evidence there is indicates that: maximum activity in these species is distributed differently over the season, L. nigripes showing an earlier peak; immatures (only 5 total were caught) may tend to be more diurnal than their adult counterpart. O. pictus showed greater flexibility than L. nigripes: in July, peak activity coincided with reversal of diel habits: minimum temperatures were as low as 36° and 52°F during two of the 24-hr trapping periods, and L. nigripes became conspicuous by diurnal at that time (Fig. 65).

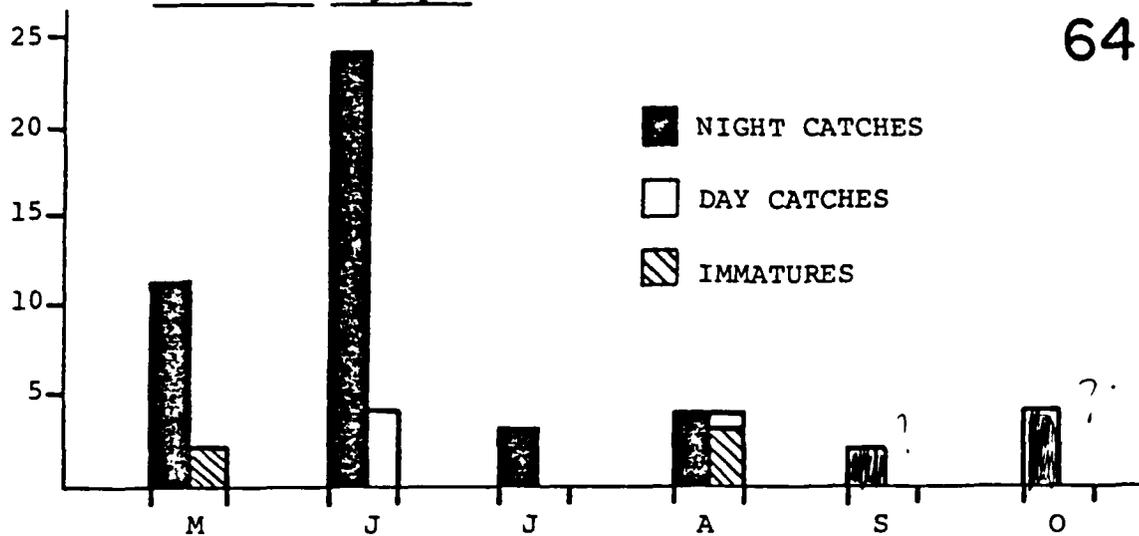
Carabidae: Carabidae, one of 23 families of Coleoptera trapped at Turner Rd., constituted over 50% of the total number caught. Sixteen species were present. Shannon-Weaver diversity indices calculated for nocturnal and diurnal species spectra were virtually equal (H' night=0.722; H' day=0.764). Five species were captured in large enough numbers for further analysis (Table 22); all belong to the tribe Pterostichini and are common in forests; P. melanarius also frequents fields and arable land (Barlow 1970; Ericson 1977).

Table 22. Total numbers of carabids, by species, trapped at Turner Rd. in 1982 and 1983.

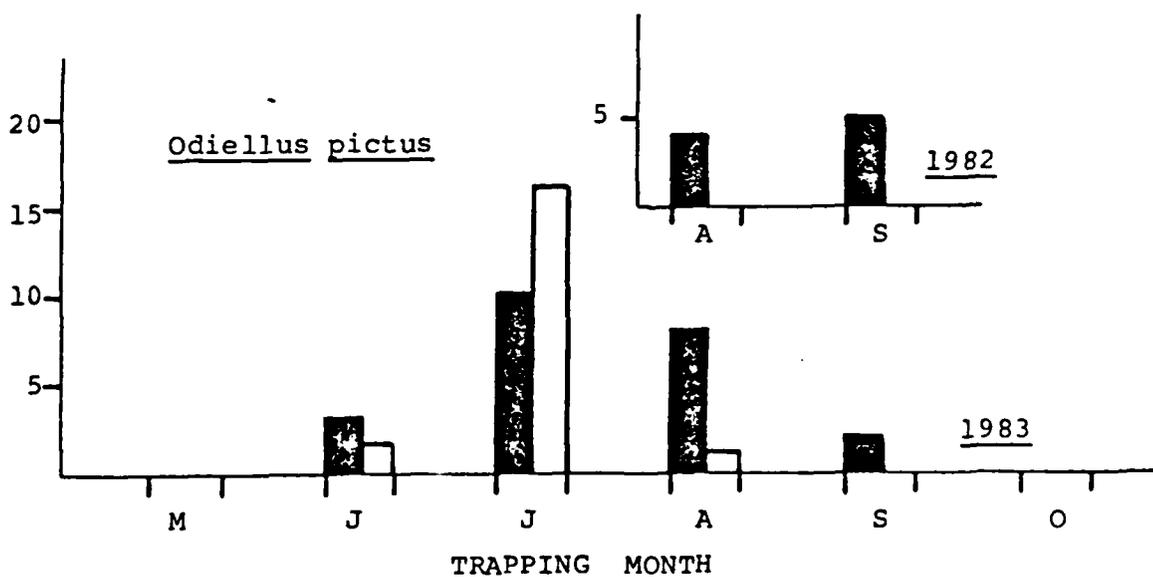
SPECIES	NO. TRAPPED	
	1982	1983
<u>Pterostichus pennsylvanicus</u>	12	215
<u>P. coracinus</u>	32	78
<u>P. melanarius</u>	16	81
<u>P. adstrictus</u>	-	5
<u>P. adoxus</u>	-	1
<u>Calathus ingratus</u>	12	156
<u>C. gregarius</u>	2	-
<u>Synuchus impunctatus</u>	27	87
<u>Cymindis cribricollis</u>	-	6
<u>Harpalus fuliginosus</u>	-	7
<u>Agonum retractum</u>	-	8
<u>A. placidum</u>	-	1
<u>Clivina fossor</u>	-	1
<u>Myas cyanescens</u>	-	1
<u>Bembidion quadrimaculatum oppositum</u>	-	1
<u>Trechus obtusus</u>	1	-

Leiobunum nigripes (1983)

64



65



Figs. 64 - 65. Total monthly catches (n = 12 traps, 1982; n = 20 traps, 1983) of the dominant opilionids at Turner Rd.

Late-season 1982 data essentially confirmed observations made in 1983. The following discussion will be based mainly on the latter.

Overall mean diurnal and nocturnal catches differed significantly for each species (Table 23), as expected. Seasonal activity maxima were made apparent by lumping trap catches into monthly totals (Fig. 66). P. pennsylvanicus exhibited two maxima; from July 21 to August 18, 1983, the species disappeared completely (lumped catches mask this hiatus). In the fall, teneral adults were numerous. Thus P. pennsylvanicus breed in the spring, their offspring mature in the fall and overwinter. Autumn activity peaks were due to new adults searching for food and hibernation sites; in agreement with Barlow (1970) we postulate that the midsummer decline may be interpreted as due to mortality, not low activity.

The remaining four species (Fig. 66) exhibited a single activity peak, temporally most narrow in S. impunctatus; i.e. the species was essentially restricted to July and August (first catch June 30, last catch September 1, 1983).

Table 23. Mean diurnal and nocturnal catches, Turner Rd. 1983, of five common carabids.

SPECIES	Mean + SE	
	NIGHT	DAY
<u>P. pennsylvanicus</u>	6.36 + 0.28	2.44 + 0.12
<u>P. coracinus</u>	2.44 + 0.11	0.56 + 0.04
<u>P. melanarius</u>	2.20 + 0.10	1.12 + 0.09
<u>C. ingratus</u>	5.92 + 0.38	0.32 + 0.03
<u>S. impunctatus</u>	3.00 + 0.23	0.88 + 0.07

All means significantly different (night vs. day) at $P < 0.01$.

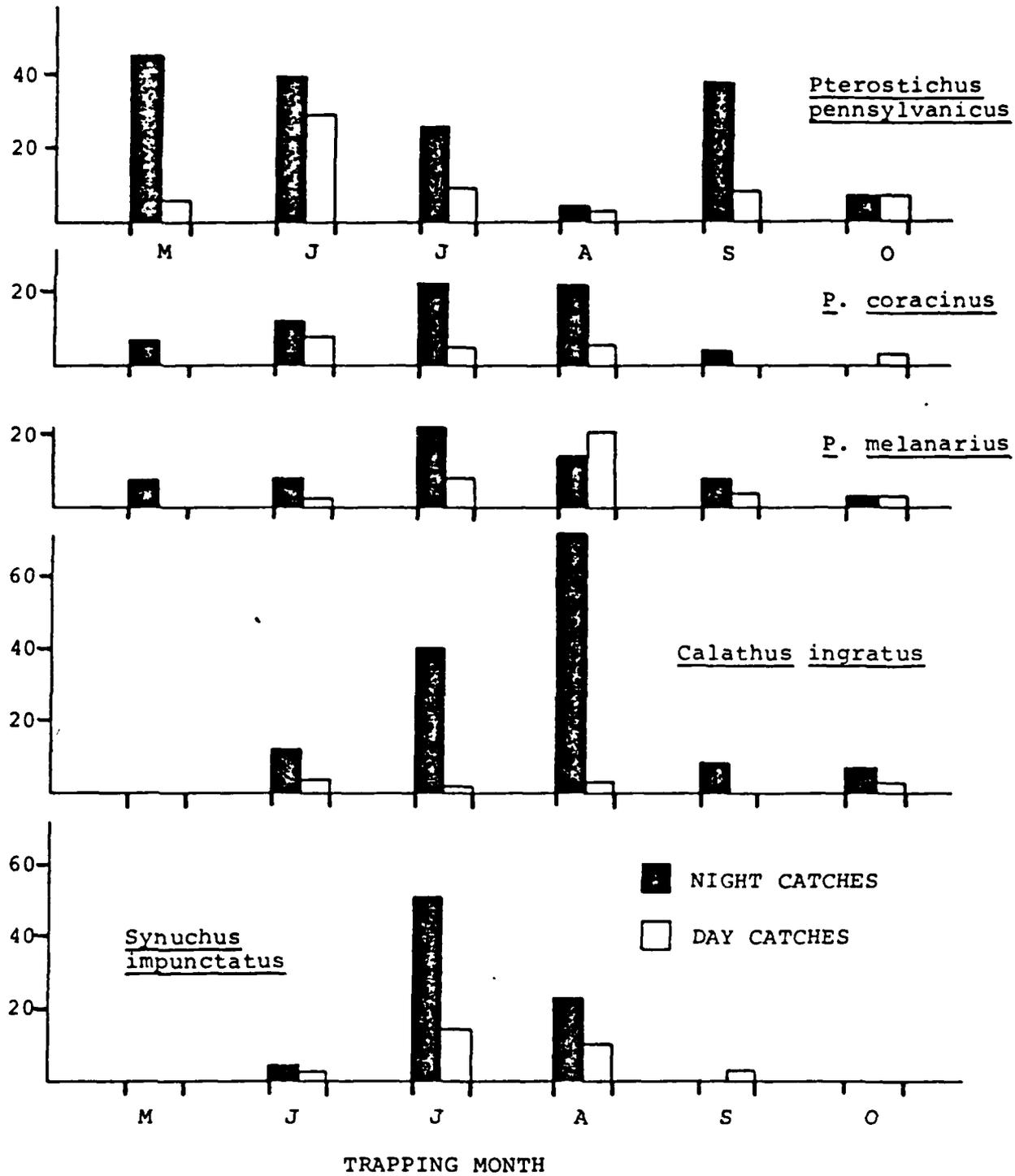


Fig. 66. Monthly diel catches (n = 20 traps) of 5 carabid species common at Turner Rd, 1983.

All four species, with teneral adults appearing in midsummer, are probably late-summer breeders. An unknown proportion of post-reproductive adults overwinter (possibly none in C. ingratus and S. impunctatus), although these populations overwinter primarily as larvae. These interpretations are in good accordance with phenological data available in the literature (Lindroth 1969; Greenslade 1965; Barlow 1970; Desender and Maelfait 1982).

P. pennsylvanicus, the only known spring breeder in the Turner Rd assemblage, may be more cold-tolerant than its associates. Indeed, all species in this Upper Peninsula site may be relatively tolerant to low temperatures. Dennison and Hodkinson (1983) were able to show that night/day activity ratios were seasonal, i.e. that carabids tended to be more day-active in spring and fall when night temperatures were low. At Turner Rd, using min/max temperatures recorded at a nearby weather station, no such trends emerged, i.e., Michigan's more severe continental temperature regime did not produce activity patterns comparable to England's mild maritime climate (Dennison and Hodkinson 1983). However, these conclusions are highly tentative and need to be tested in essentially two ways: trapping prior to May and past October, combined with on-site temperature recordings on the forest floor.

Body sizes of dominant species varied by a factor of 2, P. melanarius being approximately twice as large as C. ingratus. According to Luff (1983), large size and strong nocturnal tendency are positively correlated. Within the five species at Turner Rd., the reverse was indicated (Fig. 67): smaller species exhibited a greater degree of nocturnalism than larger ones, although the

correlation was not significant. We may be able to substantiate this weak relationship if we can document activity patterns of species other than the five common at Turner Rd.

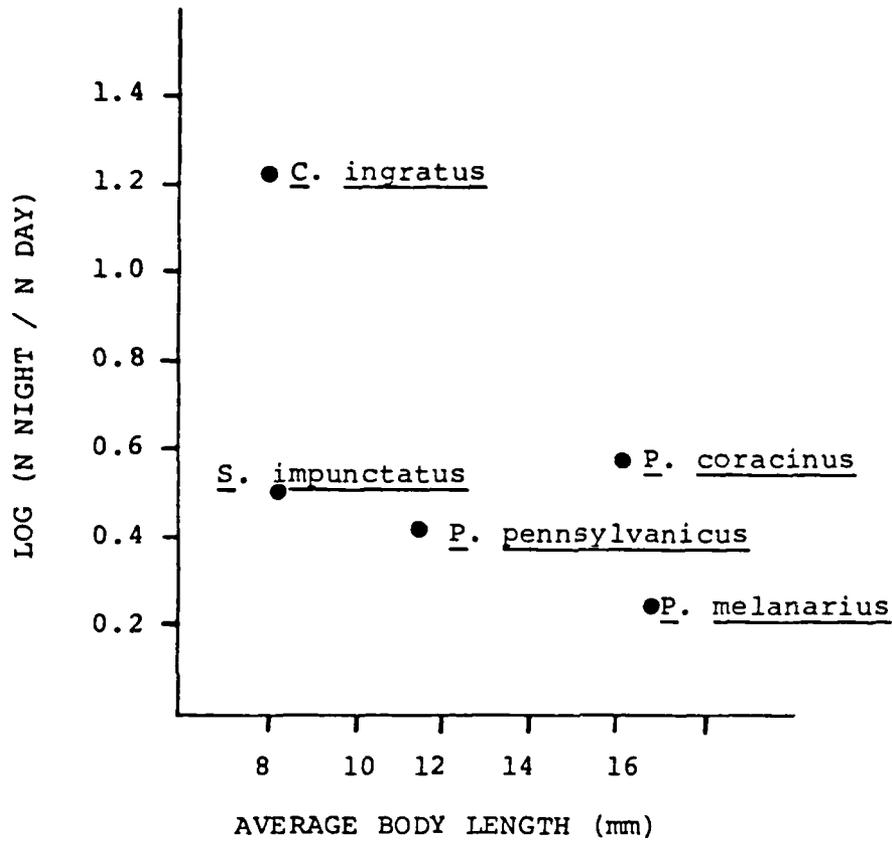


Fig. 67. Log of ratio (night catches/day catches) versus average body length of 5 carabid species common at Turner Rd.

F. Lumbricidae1. Introduction

Exotic Lumbricidae dominate the earthworm fauna of the northern United States, since endemic species were eradicated from this region by glaciation during the quaternary era (Gates, 1929). Surviving endemics in the southern United States dispersed slowly into northern states after the glaciers withdrew, but have not reached the Upper Peninsula of Michigan. The earthworm fauna of the northern United States is now dominated by peregrine species, i.e. those which are transported, usually unintentionally, by human activities: deposition of ballast from ships, importation of unsterilized soil or of potted plants, etc. (Gates 1972).

European peregrine species have been established in virtually every temperate climate on earth: New Zealand (Martin 1977), South Africa (Ljungstrom 1972), Argentina (Ljungstrom et al. 1973), Australia (Abbott and Parker 1980), Canada (Reynolds, 1977) and the United States (Gates, 1982). Indeed, all species so far collected in Dickinson Co. are european peregrines (Gates 1976): Eisenia rosea (Savigny), only rarely encountered; Dendrobaena octaedra Savigny, Aporrectodea turgida Eisen, A. tuberculata Eisen, A. longa Ude, Octolasion tyrtaeum Savigny, Lumbricus rubellus Hoffmeister, and L. terrestris L. Because of their provenance from Europe and their widespread geographic distribution, international literature is applicable to these species.

Lumbricids have been classified into guilds, a guild being defined as a group of species that exploits the same class of

environmental resources in a similar fashion (Price, 1978). Bouche (1977) divided european peregrine earthworms into three basic guilds: epiges, endoges, and aneciques. Epiges are characterized by: living in litter, small size, high reproductive rates, short life spans, and dark pigmentation. Of the species we deal with, L. rubellus and D. octaedra are epiges. Endoges are of moderate size, live in and feed on soil, have moderate life spans, and are unpigmented. A. turgida, A. tuberculata, and O. tyrtaeum are endoges. Aneciques typically have low reproductive rates, live in deep permanent burrows, feed on litter, and possess long life spans, dark pigmentation, and large size. L. terrestris and A. longa are aneciques. In the present report, these guild classifications will be used as a unifying theme for the discussion of data obtained on lumbricid populations in the ELF system area.

Once the definitive antenna corridor became known (spring 1983), Task 5.4. (Lumbricidae) joined its companion (5.3., Arthropoda) in searching for ecologically well-matched Test and Control sites. During the preceding 1982 half-season, as well as during 1983, a temporary study site (Turner Rd) was used to prepare for full-scale lumbricid research in definitive sites. The Turner Rd. site is very similar to Test and Control. It harbors several of the same species, so that the site provided a preliminary understanding of lumbricid population characteristics in the ELF system area.

Below, we describe major methodological modifications, and their validation, arrived at in 1983. Using all data available at this time, we then quantify lumbricid population parameters in Test and Control sites, and finally present the supportive data set obtained at Turner Rd.

2. Technique optimization and validation

Earthworm sampling techniques fall into two basic categories: handsorting and formalin extraction. In formalin extraction, a solution of .00625% formalin is applied to a quadrat of known area (Raw, 1959), causing the worms to come to the soil surface in an effort to escape the formalin's irritation. The method is quick, can be applied to large quadrats of 1 m², and is especially efficient for sampling large, permanent-burrow inhabiting species (aneciques). However, since only active earthworms are recovered, the efficiency of formalin extraction varies through the year (Bouche' 1969), and shallow-burrowing forms often respond poorly. For these reasons, the technique was not considered adequate for the purposes of this study.

Handsorting consists of carefully searching through a given amount of soil for the presence of worms; according to Zicsi (1962), 0.0625 m² represents an optimal sample size. Recovery of specimens by this method is not dependent on worm activity, and there is no chemical (formalin) disturbance of the sampled area. The trade-off, however, is that one cannot dig deep enough to include all of the deep-burrowing aneciques (Terhivuo, 1982). Overall, handsorting is highly reliable, yielding 95% of total worm biomass and 80% of total worm numbers (Axellson et al, 1971), but recovery of small, dark worms (epiges) is poor (Raw, 1960). D. octaedra, found in large numbers in both Test and Control sites, falls into that category. The technique also recovers only low percentages of cocoons, which are effectively invisible unless the soil capsule surrounding them is broken. Finally, the third drawback of handsorting is its tediousness. Even highly motivated

sorters will suffer an occasional lapse in attention and overlook earthworms. It was therefore highly desirable to use the handsorting method, but also to modify it in a way that would recover cocoons and small as well as missed earthworms.

Satchell (1969) thoroughly reviewed earthworm sampling techniques, but did not mention cocoon recovery; neither did two recent articles by Terhivuo (1982) and Springett (1981). Raw (1960) improved recovery of small, dark earthworms by washing handsorted soil through a sieve, and suggested that the method might be modified to yield cocoons. Gerard (1967) estimated cocoon density in the field through wet sieving, but did not publish any information on extraction efficiency.

The technique developed and validated in the present study was a two-step process designated as sorting/sieving (Fig. 68). A soil sample of .0625 m² (25 by 25 cm) was excavated and bagged in 4 subsample increments: leaf litter, humus, mineral soil 0 to 10 cm below humus, and mineral soil 10 to 20 cm below humus. Each subsample was handsorted, then wet-sieved through a 5 mm mesh which retained large stones and debris; fine residue was then washed through a 1.5 mm mesh. Worm castings and soil aggregates caught on this screen were gently broken apart in successive washings, and cocoons and worms were picked off.

There were two sources of error in this method: worms and cocoons may have passed through the 1.5 mm sieve, or may have been overlooked. Validation was therefore performed on 32 humus samples taken from Turner Road and Test (Fig. 68). All residues of these samples were passed through an additional 1.0 mm mesh sieve, and

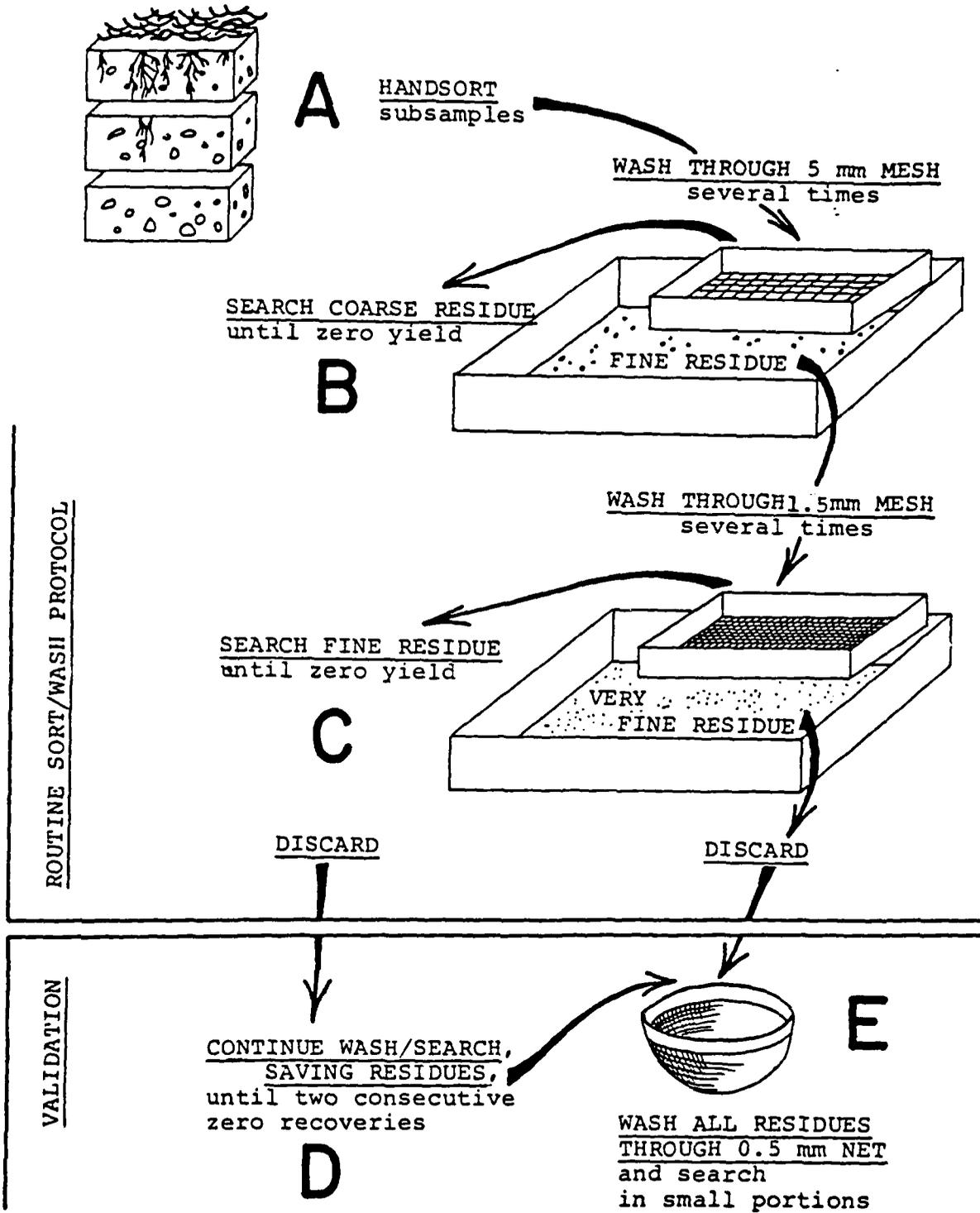


Fig. 68. Stepwise diagram of wet-sieving technique adopted for sampling lumbricid populations.

the residue remaining on the 1.5 mm sieve was rewashed until no worms or cocoons were found on 2 successive rewashings (Fig. 68).

Tables 1-5 show results of the validation study for 5 species of earthworms. Handsorting alone recovered the majority of the worms, but only 11.6% of the cocoons. The sorting/sieving combination yielded excellent results, recovering 97.7% of the worms and 96.7% of the cocoons. Virtually no cocoons passed through the 1.5 mm sieve (almost all missed cocoons were still on the 1.5 mm sieve but had been overlooked). Some worms were overlooked and some passed through the 1.5 mm sieve. The worms have not yet been weighed, but the biomass recovery rate is expected to exceed 99%. Sorting/sieving requires an average of 90 minutes per sample (humus and mineral soil).

The new technique was less than satisfactory for recovering worms from leaf litter, which proved laborious to handsort and difficult to sieve. A modified formalin technique was therefore perfected for litter samples. They were moistened and placed into plastic bags for 1 day so that dessicated worms would become active. Each sample was then suspended 5 cm from the bottom of an 8 liter bucket, on a 10 mm mesh sieve. Six liters of .025% formalin were added, causing the worms to become hyperactive and move from the litter into the solution below. After 1 hour, the formalin solution was poured through a 1 mm mesh sieve. Worms were easily picked off it and preserved.

The method was validated as follows: litter was dried to kill any worms which may have been in it. It was then moistened and 40 worms were added. After 24 hours, formalin extraction was performed

Tables 24 through 28. Species-specific percentages of worms and cocoons recovered the sort/sieve method. For description of steps A-D, ref. to Fig. 67.

24. Dendrobaena octaedra

Process	STAGE		
	Cocoons	Immatures	Adults
Handsort (A)	14.4	90.5	98.6
Wet Sieve 1.5 mm mesh (B)	84.1	6.3	1.4
Wash 1.5 mm sieve (C)	1.5	1.0	0.0
Wet Sieve 1.0 mm mesh (D)	0.0	2.2	0.0
Percentage missed (C + D)	1.5	3.2	0.0
Percentage found (A + B)	98.5	96.8	100.0
Total numbers (A+B+C+D)	659	411	73

25. Octolasion tyrtaeum

Process	STAGE		
	Cocoons	Immatures	Adults
Handsort (A)	11.7	90.0	100.0
Wet Sieve 1.5 mm mesh (B)	86.9	6.9	0.0
Wash 1.5 mm sieve (C)	1.2	2.5	0.0
Wet Sieve 1.0 mm mesh (D)	0.2	0.6	0.0
Percentage missed (C + D)	1.4	3.1	0.0
Percentage found (A + B)	98.6	96.9	100.0
Total numbers (A+B+C+D)	486	158	58

26. Lumbricus rubellus

Process	STAGE		
	Cocoons	Immatures	Adults
Handsort (A)	1.6	90.0	100.0
Wet Sieve 1.5 mm mesh (B)	84.1	7.6	0.0
Wash 1.5 mm sieve (C)	1.2	1.5	0.0
Wet Sieve 1.0 mm mesh (D)	12.8	1.5	0.0
Percentage missed (C + D)	14.3	3.0	0.0
Percentage found (A + B)	85.7	97.0	100.0
Total numbers (A+B+C+D)	188	66	9

27. Aporrectodea tuberculata

Process	STAGE		
	Cocoons	Immatures	Adults
Handsort (A)	12.5	90.1	100.0
Wet Sieve 1.5 mm mesh (B)	81.2	9.9	0.0
Wash 1.5 mm sieve (C)	6.3	0.0	0.0
Wet Sieve 1.0 mm mesh (D)	0.0	0.0	0.0
Percentage missed (C + D)	6.3	0.0	0.0
Percentage found (A + B)	93.7	100.0	100.0
Total numbers (A+B+C+D)	16	71	8

28. Aporrectodea longa

Process	STAGE		
	Cocoons	Immatures	Adults
Handsort (A)	-	100.0	100.0
Wet Sieve 1.5 mm mesh (B)	-	0.0	0.0
Wash 1.5 mm sieve (C)	-	0.0	0.0
Wet Sieve 1.0 mm mesh (D)	-	0.0	0.0
Percentage missed (C + D)	-	0.0	0.0
Percentage found (A + B)	-	100.0	100.0
Total numbers (A+B+C+D)	0	14	2

as described above, yielding 96 ± 1.0 (std. err. of mean, $n=16$) percent of the worms. The method was rapid, taking only 15 minutes/sample, and provided considerable aid in assessing epigeic species populations.

Thus the sort/sieve and litter extraction procedures optimized during 1983 ensure accurate sampling of worms of all size classes as well as of cocoons. Small worms are missed in many sampling systems, and recovery of cocoons is usually so poor that their numbers are not reported at all. Considering that lumbricid reproductive rate is a likely population parameter to be affected by ELF radiation, accurate estimation of both cocoon and immature worm density is of prime importance in the present study.

3. Sampling schedule and methods:

On each date (Table 29), twenty $1/16 \text{ m}^2$ samples were taken and handsorted. 1982 Turner Rd. samples consisted of humus, 0-10 and 10-20 cm below humus layers (1982 litter subsamples were used to heat - extract arthropods). All 1983 samples, however, included a litter increment. The sort/sieve method for humus and soil was finalized in July and implemented in August of 1983; formalin extraction of litter was initiated on October 10 and used on all dates thereafter.

Table 29. Earthworm sampling schedule 1982-83.

<u>1982</u>											
Turner Rd.	12/VIII	20/VIII	8/IX								
<u>1983</u>											
Turner Rd.	8/V	23/V	9/VI	20/VI	5/VII	18/VIII	1/VIII	15/VIII	6/IX	26/IX	17/X
Test	-	-	-	-	-	-	8/VIII	24/VIII	11/IX	3/X	24/X
Control	-	-	-	-	-	-	9/VIII	22/VIII	14/IX	10/X	31/X

The thickness of humus subsamples was recorded at the time they were dug in the field. In the fall of 1983, litter subsamples, following formalin extraction of lumbricids, were used to assess litter standing crops in Test and Control.

Prior to implementation of the sort/sieve method, sample holes in the field were refilled with humus and soil subsamples after these had been sorted. Wet-sieving, however, precluded returning samples to the field. A "borrow pit" was therefore established just outside the boundaries of each site, and sample holes were refilled with soil taken from these pits. They, in turn, were filled in at the close of the season with soil which had accumulated at the site where wet-sieving had been performed.

4. Results

4.1 Lumbricid sample distributions:

In order to describe sample distributions of all species commonly encountered so far, data from the following sites and dates were used:

Turner Rd. 1982 (Aug. 12, 20; Sept. 9): A. tuberculata, O.

tyrtaeum, D. octaedra, L. rubellus;

Control site (Aug. 8; Sept. 11): A. turgida.

Observed frequency distributions of these species were fitted to negative binomial (aggregated), Poisson (random) and Normal (continuous) distributions. Subjected to a Chi-square goodness of fit test, four of the five conformed to the negative binomial, one to the Normal as well as the negative binomial, and none to the

Poisson distribution (Table 30). In Figures 69-73, comparisons between actual frequency distributions and the expected negative binomial are shown graphically, and the value of K is given for each species. K, one of the determinants of the negative binomial, can also be used as an index of aggregation (Southwood 1978): the smaller K, the greater the degree of clumping. Thus A. tuberculata is highly aggregated, A. turgida least so; indeed, frequency distribution of A. turgida did not deviate significantly from normality.

As a result of clumping, most of our earthworm data can only be tested by parametric statistics (t-test, ANOVA) after log transform (Elliott, 1971).

Table 30. Results of the Chi-square goodness of fit test of observed frequency distributions in 5 lumbricids (n.s. = not significant; values <0.05 indicate significant deviation from expected distributions).

<u>Species</u>	<u>Distribution</u>		
	<u>Negative Binomial</u>	<u>Normal</u>	<u>Poisson</u>
<u>A. turgida</u>	n.s	n.s	.005
<u>A. tuberculata</u>	n.s	.001	.0001
<u>O. tyrtaeum</u>	n.s.	.005	.0001
<u>L. rubellus</u>	n.s.	.0001	.0001
<u>D. octaedra</u>	n.s.	.005	.0001

NEG. BIN. DISTRIBUTION FIT
L. RUBELLUS

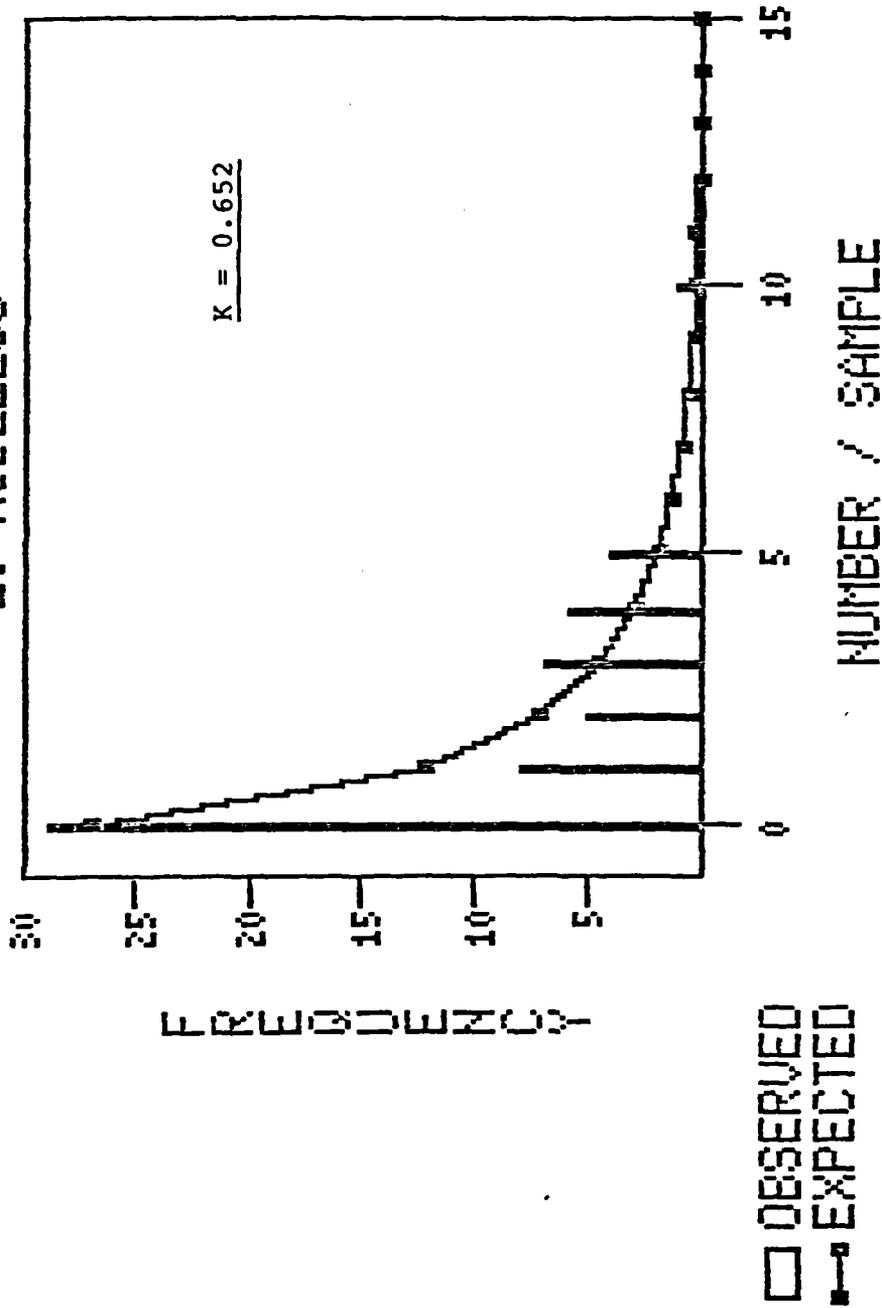


Fig. 69. Sample distribution of Lumbricus rubellus compared to the negative binomial.

NEG. BIN. DISTRIBUTION FIT
D. OCTAEDRA

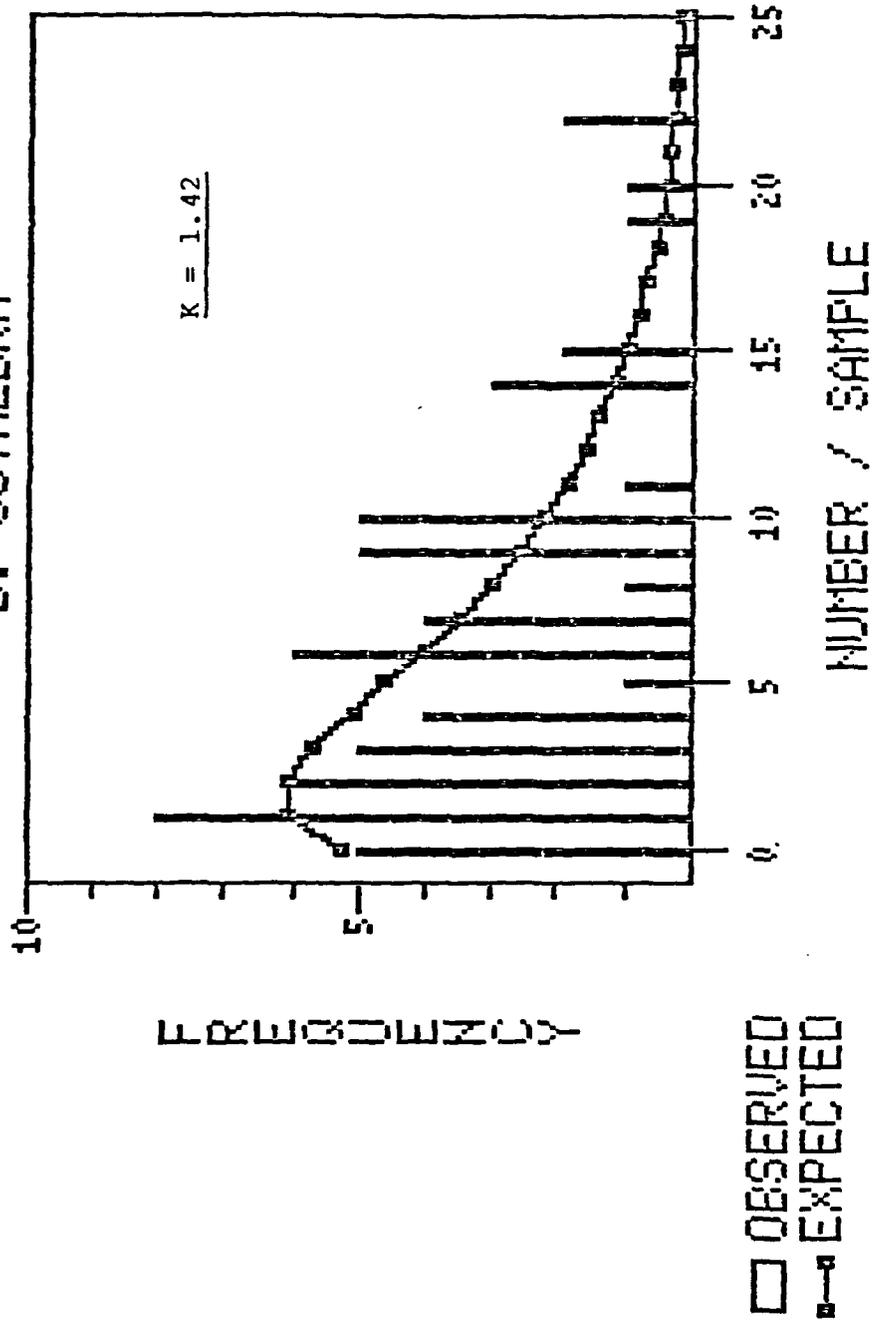


Fig. 70. Sample distribution of Dendrobaena octaedra compared to the negative binomial.

NEG. BIN. DISTRIBUTION FIT
 Á. TUBERCULÁTÁ

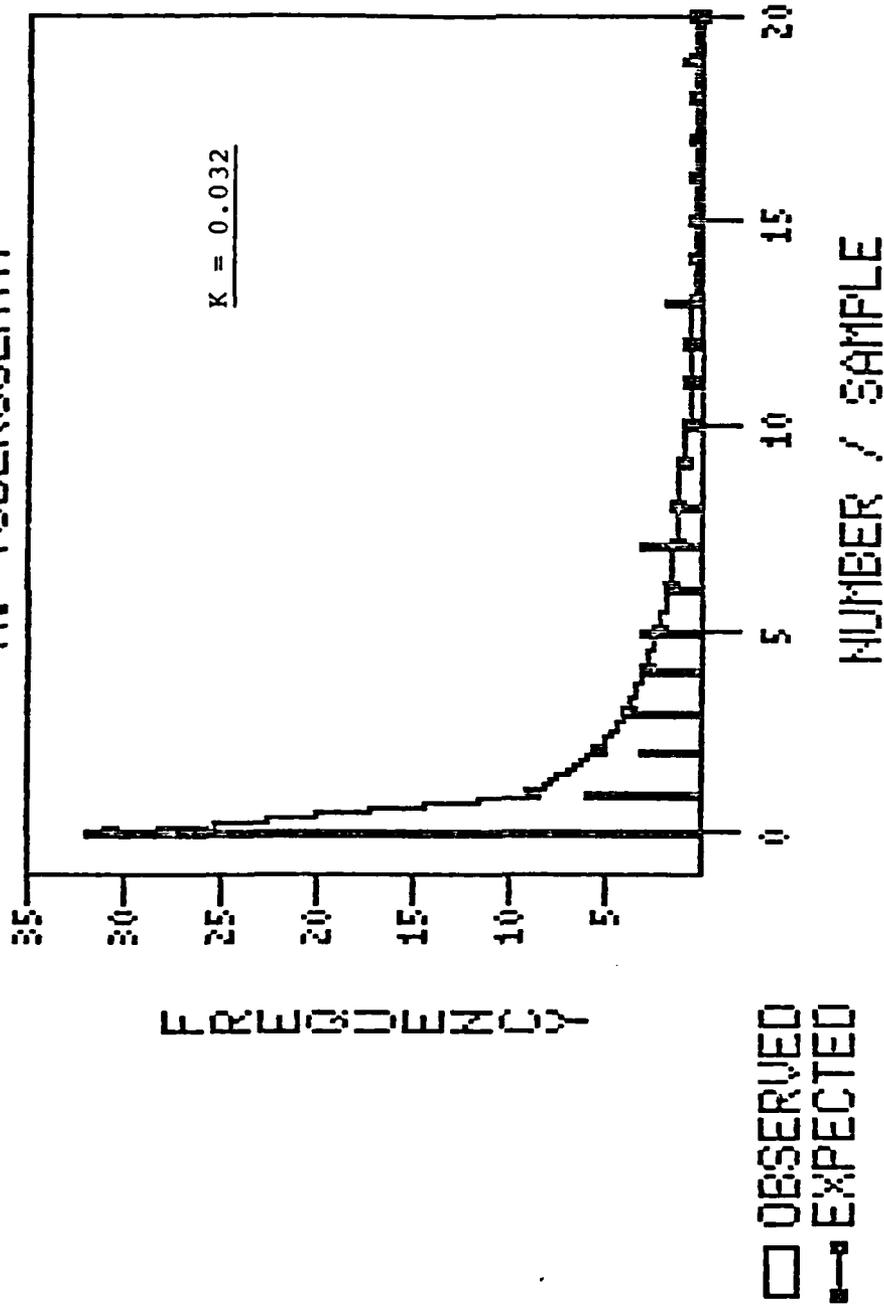


Fig. 71. Sample distribution of Aporrectodea tuberculata compared to the negative binomial.

NEG. BIN. DISTRIBUTION FIT
O. TRYTAEUM

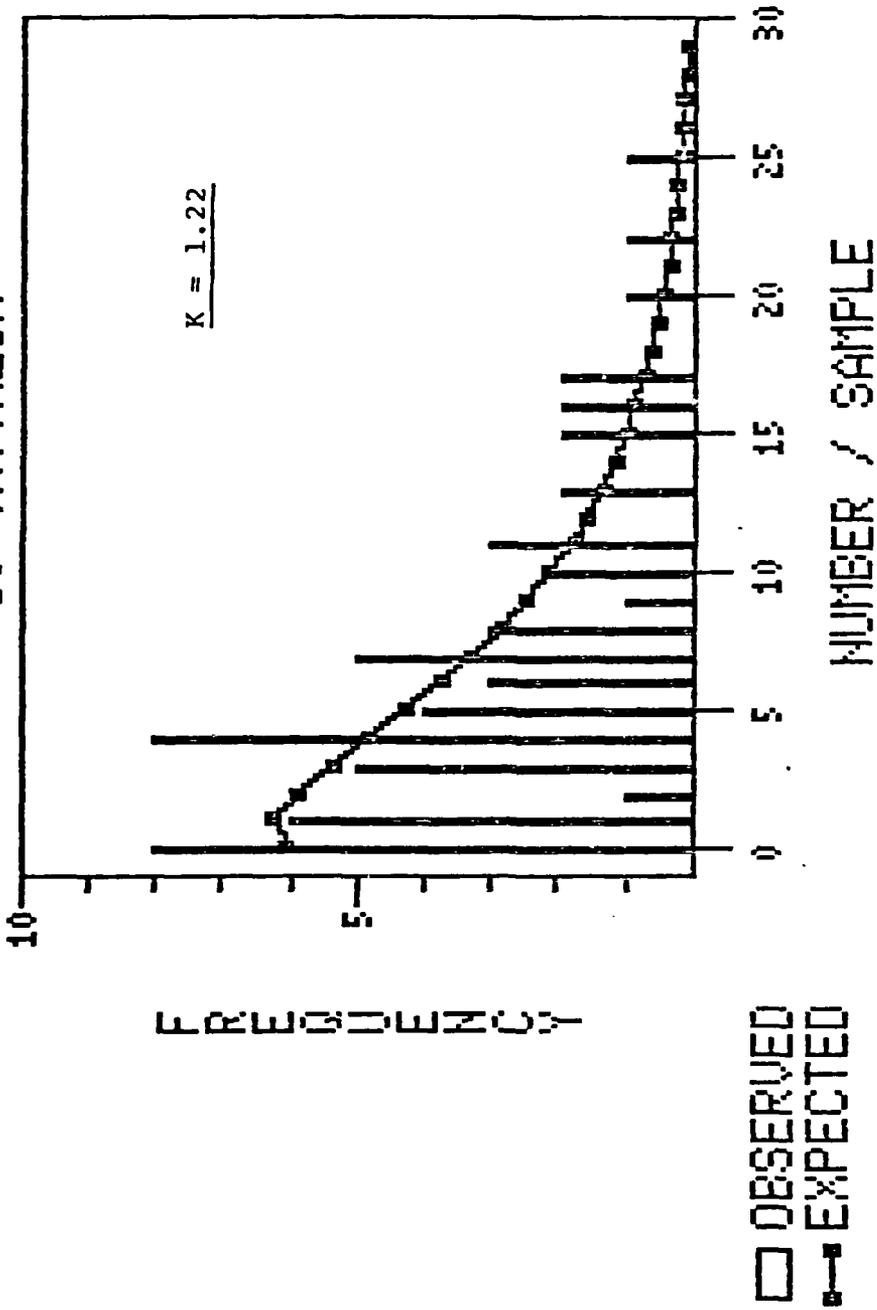


Fig. 72. Sample distribution of Octolasion tyrtaeum compared to the negative binomial.

NEG. BIN. DISTRIBUTION FIT
A. TURGIDA

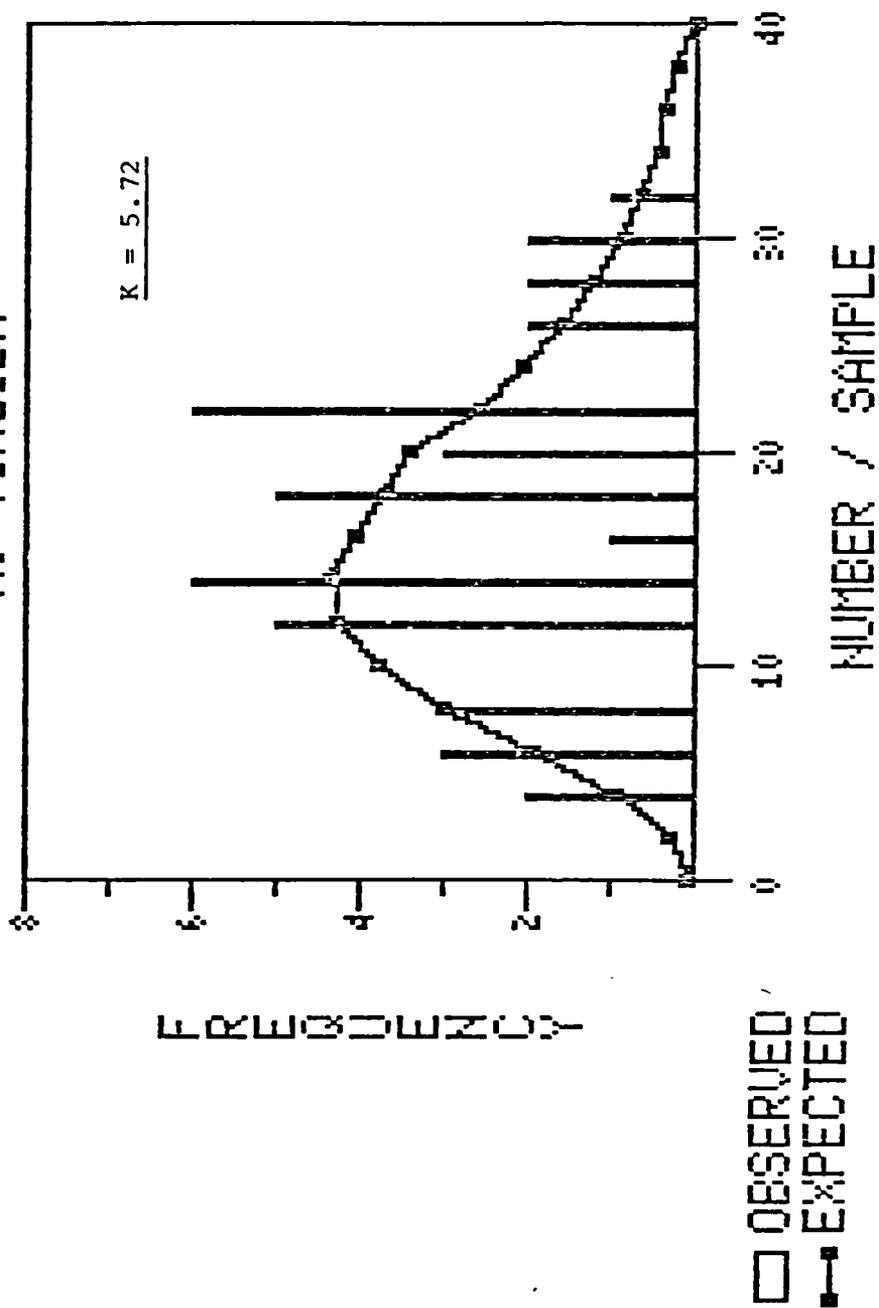


Fig. 73. Sample distribution of *Aporectodea turgida* compared to the negative binomial.

4.2 Lumbricid associations in Test and Control sites

a. Species composition, density, biomass:

Dominance relationships of species within lumbricid associations can be expressed in terms of numbers of individuals, or in terms of biomass. Using data from two sampling dates, species-specific densities and biomass in Test and Control were estimated and compared (Table 31). Major between - site differences can be summarized as follows: the Control site is dominated by A. turgida and D. octaedra (both in terms of density and of biomass), and harbors small populations of L. rubellus and A. longa. In terms of weight, however, A. longa is a subdominant. The Test site is dominated by A. tuberculata, with L. rubellus and D. octaedra being less numerous. Again, A. longa occurs in small numbers but contributes a large amount of biomass. Dominance patterns differ significantly between sites (G test, $P < 0.001$). However, total numbers and biomass are very similar even though species compositions only partly overlap: of four species occurring in each association, three are shared between sites, while the fourth (and dominant) species differ (Table 31).

With respect to these dominants (A. tuberculata in Test and A. turgida in Control) it must be emphasized that they were once considered synonyms of A. caliginosa, until Gates (1972) restored them to species status. Although some authorities would disagree, we are presently following Gates' (1972) nomenclature. It is obvious that, if we lumped A. tuberculata and A. turgida into a single species A. caliginosa, species compositions and dominance relationships (Table 31) in the two sites would be very similar.

Neither abundance nor biomass differed significantly between sites (t-test on log-transformed data). Averaged over both sites, densities were estimated at 413 individuals/m², with a biomass of 62.5 g. These populations are relatively dense compared to others reported in the literature: 165 worms/m², weighing 41 g/m², in British deciduous woods (Phillipson et al. 1978); 384/m², biomass 24 g/m², in forests in Poland (Nakamura 1975); in elm-ash and elm woods in southern Sweden, densities varied from 122-227/m², with up to 89 g/m² biomass in areas where large-bodied species dominated (Nordstrom and Rundgren 1973). As a rule, however, forests in north-temperate climates harbor large numbers of lumbricids, with densities frequently estimated at 200-300/m² (Nordstrom and Rundgren 1973; review in Satchell 1967).

Categorization according to Bouche's (1977) criteria grouped the species listed in Table 31 as follows:

- a) endoges are represented by A. tuberculata and A. turgida, both of which are good burrowers feeding below the surface
- b) epiges, i.e. species which feed on litter and make temporary, shallow burrows into the A layer, are represented by L. rubellus and the small-bodied D. octaedra;
- c) aneciques: A. longa, deep-burrowing, surface - casting, and feeding on litter, belongs in this guild.

Expressed in percent of total worms collected/site, these guilds clearly are equally distributed in Test and Control (Table 32). The relative importance of functional groups within each association is thus virtually identical.

Table 31. Comparison of numbers/m² and grams/m² (in parenthesis), \pm SE, of earthworms species at Test and Control sites on two dates in 1983.

Species	CONTROL		TEST	
	Aug. 9	Sept. 14	Aug. 8	Sept. 11
<u>A. tuberculata</u>	0.0	0.0	182 \pm 26	250 \pm 31 (47.8 \pm 5.1)
<u>A. turgida</u>	249 \pm 26	271 \pm 29 (37.8 \pm 5.8)	0.0	0.0
<u>D. octaedra</u>	135 \pm 22	151 \pm 23 (7.7 \pm 1.1)	8.8 \pm 3.0	53 \pm 15 (2.2 \pm 0.7)
<u>L. rubellus</u>	0.0	0.8 \pm 0.8 (0.7 \pm 0.4)	56 \pm 9.0	64 \pm 12 (10.4 \pm 2.2)
<u>A. longa</u>	2 \pm 1	15 \pm 4 (10.6 \pm 1.6)	20 \pm 4	22 \pm 5 (10.0 \pm 2.2)
Total numbers	386 \pm 31	437 \pm 41	267 \pm 31	389 \pm 46
(Total biomass)		(57 \pm 6.6)		(68 \pm 6.0)

Table 32. Comparison of earthworms guilds at Test and Control sites (Aug. - Sept., 1983) expressed in percentages of total earthworms.

Guild Category	CONTROL		TEST	
	numbers	weight	numbers	weight
endoges	62.0	66.1	64.2	70.7
epiges	34.7	14.6	30.1	14.5
aneciques	3.5	18.5	5.7	14.8

b. Distribution of developmental stages:

We have begun analysis of the stage structure of species populations in Test and Control, in order to arrive at estimates of population growth, extent of recruitment, and seasonal and stage-specific mortality. At this time, data from one date (September) are completed; results are presented below.

Collected specimens were initially separated into anatomically defined stages: immatures, adults, and cocoons. All available specimens were then weighed and assigned to weight classes within each life stage. We thus obtained a one-point description of weight frequencies in autumnal species populations; as further data accumulate, seasonal fluctuations in these parameters can be quantified.

A. tuberculata cocoons were rarely found, even with the sort/sieve technique, so that their weight frequencies could not be meaningfully analysed. Cocoon weights in L. rubellus (Fig. 74), D. octaedra (Fig. 75) and A. turgida (Fig. 76) all conformed to a normal distribution (Chi-square test). Weight ranges were broad in all species, the smallest (D. octaedra) producing the lightest cocoons.

Evans and Guild (1948) maintained that cocoon weights remained constant throughout the year. However, according to evidence presented by Phillipson and Bolton (1977), cocoon weights positively correlate to weight of adults that produced them. Since it is likely that cocoon weights do not change during development (Evans and Guild 1948; Phillipson and Bolton 1977), a possible relationship between seasonally variable state of adult development and cocoon weight will need to be further investigated.

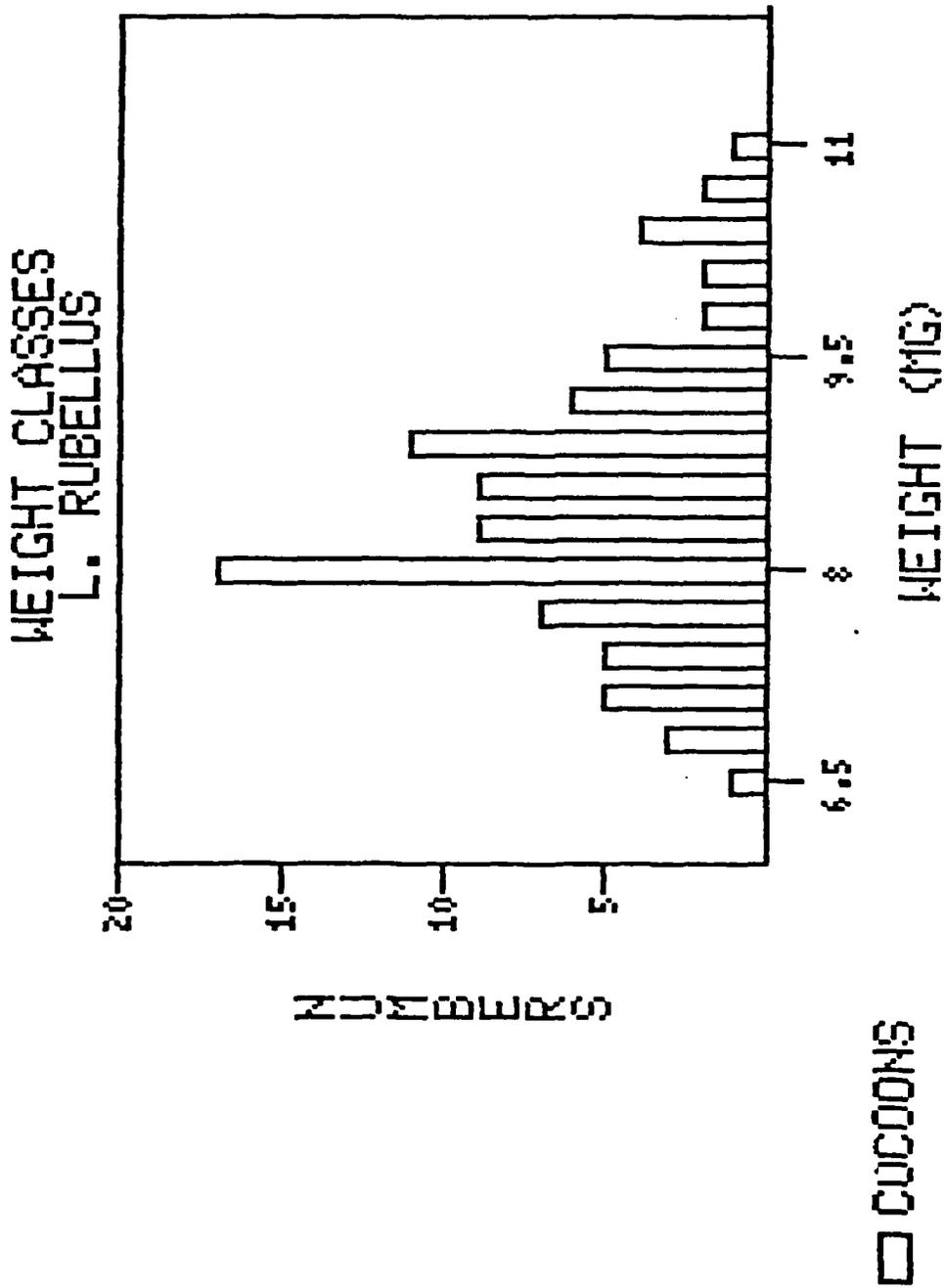


Fig. 74. Frequency distribution of cocoon weights of Lumbricus rubellus.

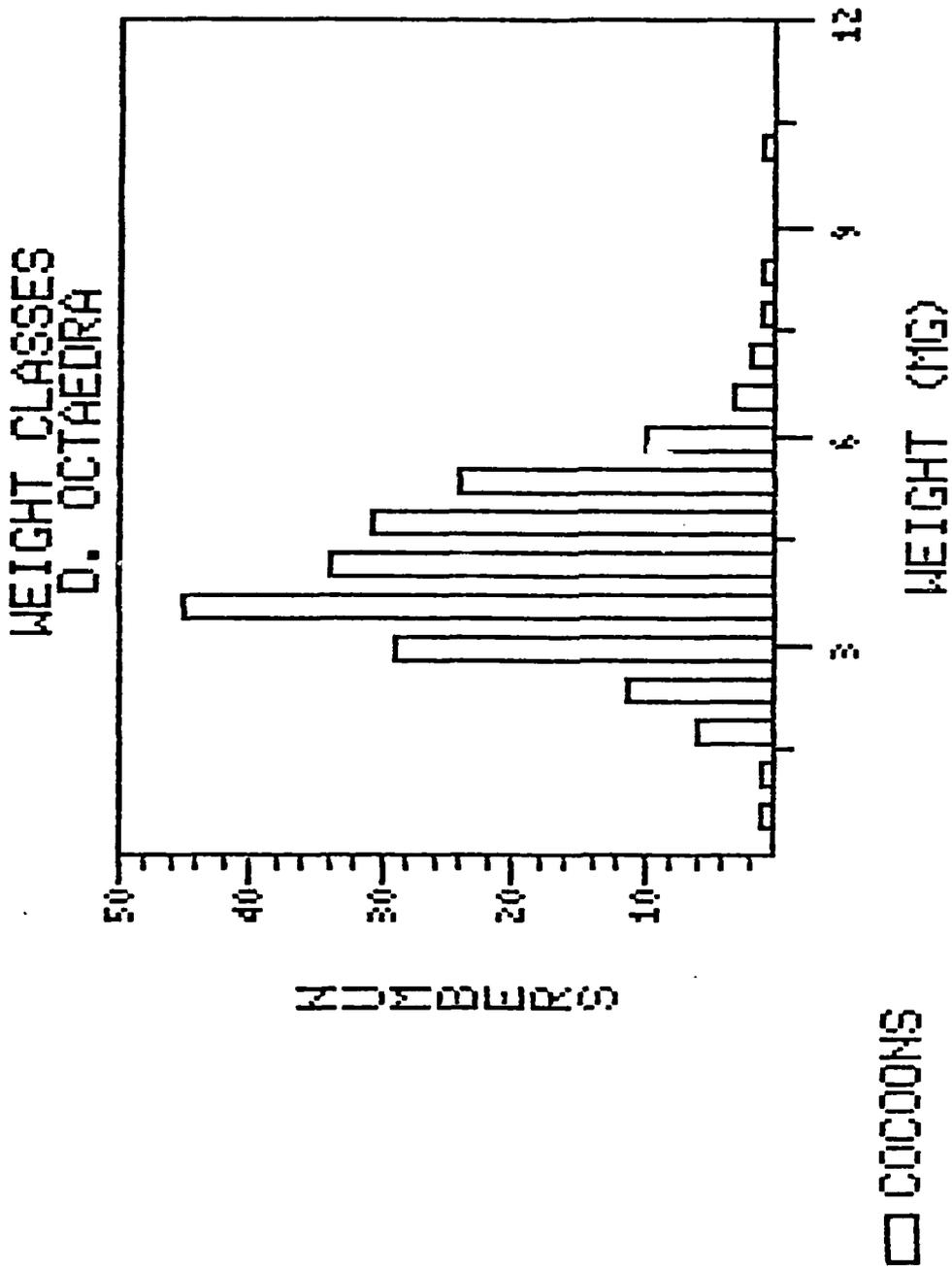


Fig. 75. Frequency distribution of cocoon weights of Dendrobaena octaedra.

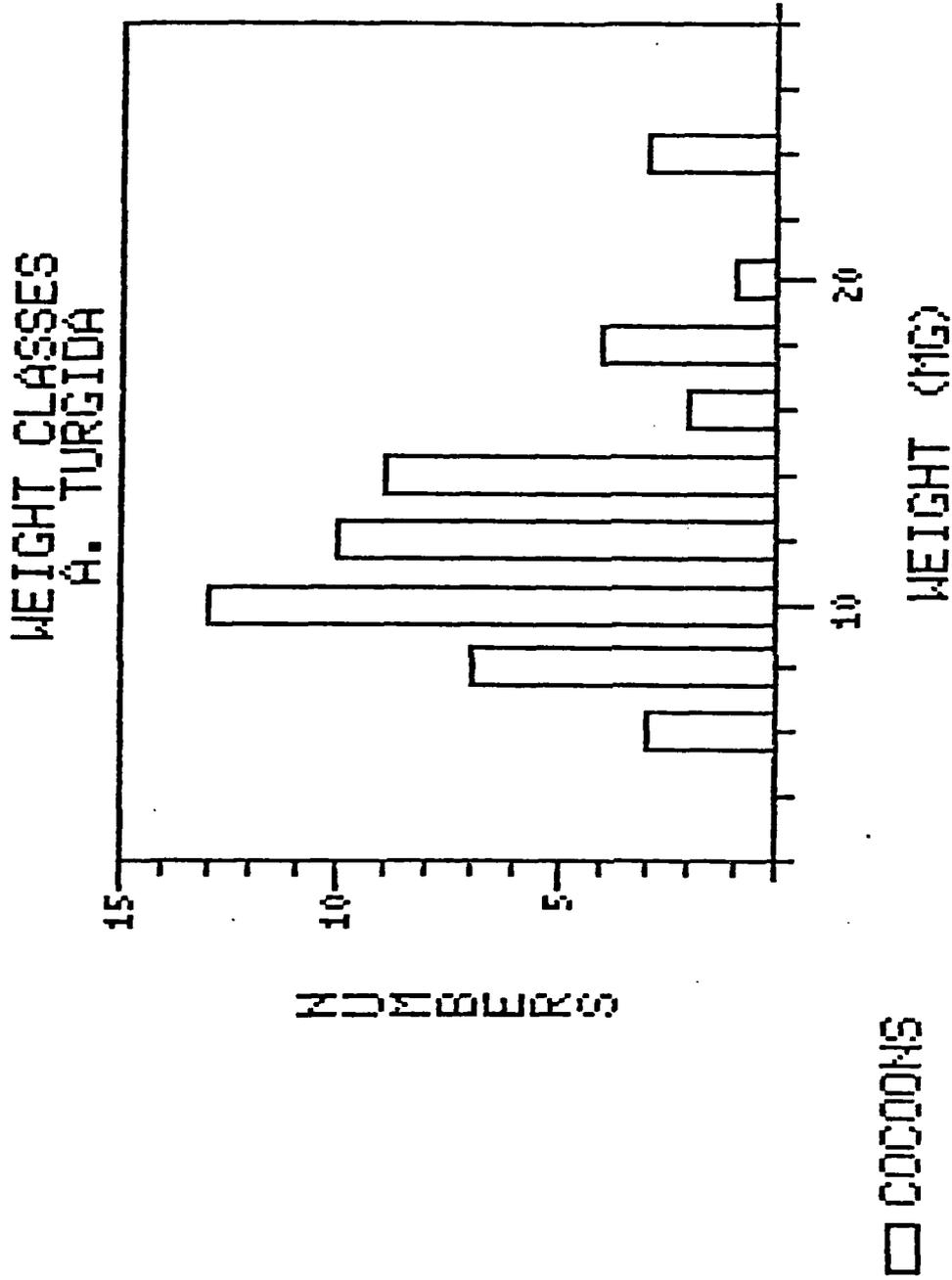


Fig. 76. Frequency distribution of cocoon weights of Aporectodea turgida.

Within immatures, the lowest weight classes were by far the most frequent in all species (Figs. 77-80). Given that growth rates of immature lumbricids tend to be rapid and linear (Neuhauser et al. 1979; Mazantseva 1982; Tsukamoto and Watanabe 1977), rapid decrease in frequencies of large immatures (Figs. 77-80) indicates high mortality of small (young) worms.

Immature and adult weights overlapped somewhat in all species (Figs. 77-80). Keeping in mind that September populations reflect the summed influences of environmental effects prior to the sampling date, adult development could be used to deduce species-specific adaptation as follows:

Adults of L. rubellus, A. turgida and A. tuberculata tended to possess faintly developed, non-functional clitella, i.e. they were not producing cocoons at the time. D. octaedra, on the other hand, typically had swollen, glandular clitella indicative of cocoon production. All four species are potentially able to reproduce year-round (Reynolds 1977). Only the smallest epige (D. octaedra), however, seems to rapidly take advantage of propitious changes in its environment: reproduction occurs in direct and immediate response to increased litter moisture following even a slight increase in precipitation. The other three species were still in the non-reproductive state which, in lumbricids, is induced by high temperatures and/or low soil moisture during the summer months (Gerard 1967). Soil-dwellers in particular (A. tuberculata and A. turgida) are probably dependent on prolonged or heavy rains able to penetrate litter and humus; as evidenced by soil suction records obtained in Test and Control in late summer this did not happen in

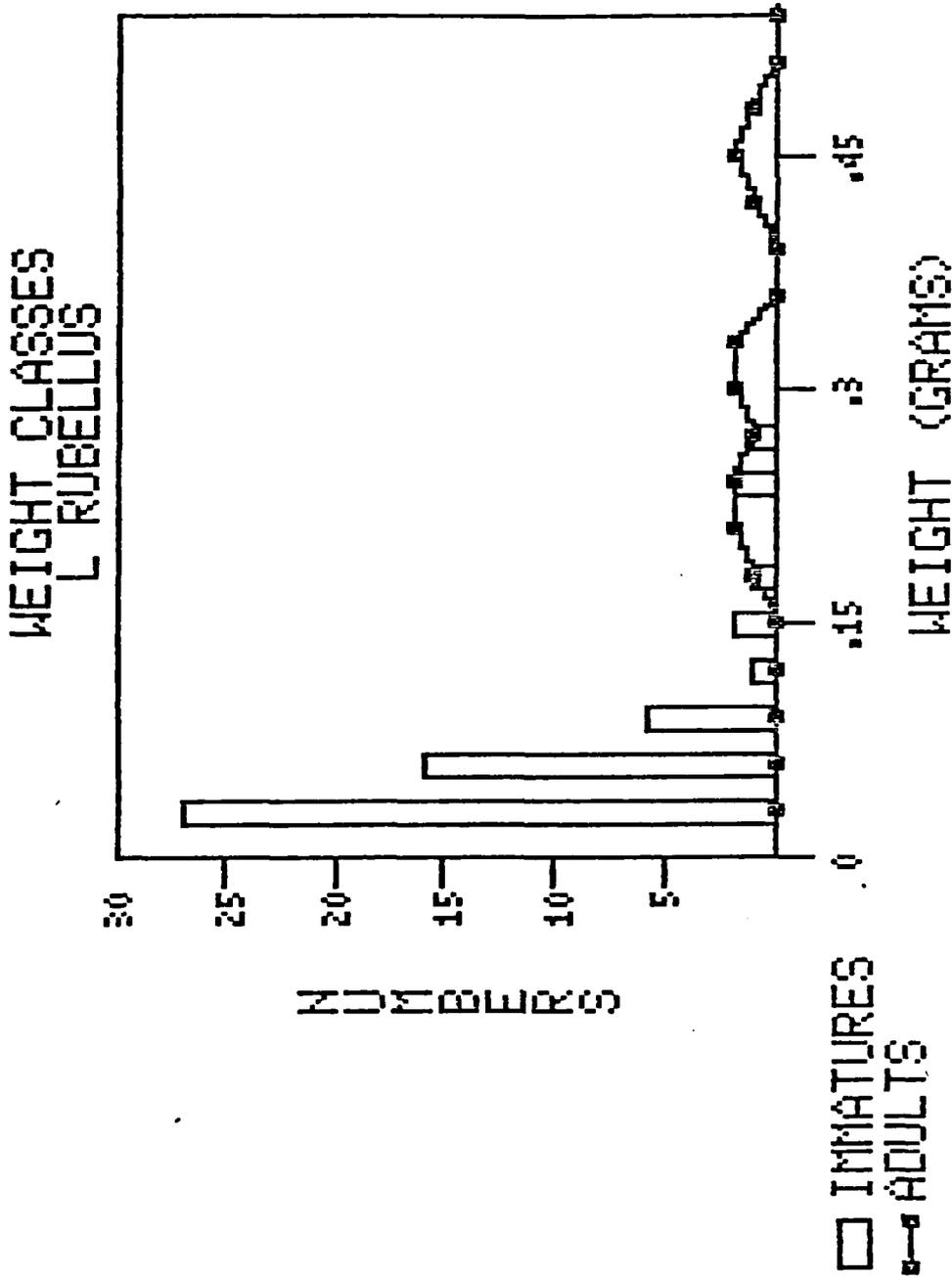


Fig. 77. Weight frequencies in September populations of Lumbricus rubellus (combined Test and Control samples), 1983.

WEIGHT CLASSES
D. OCTAEDRA

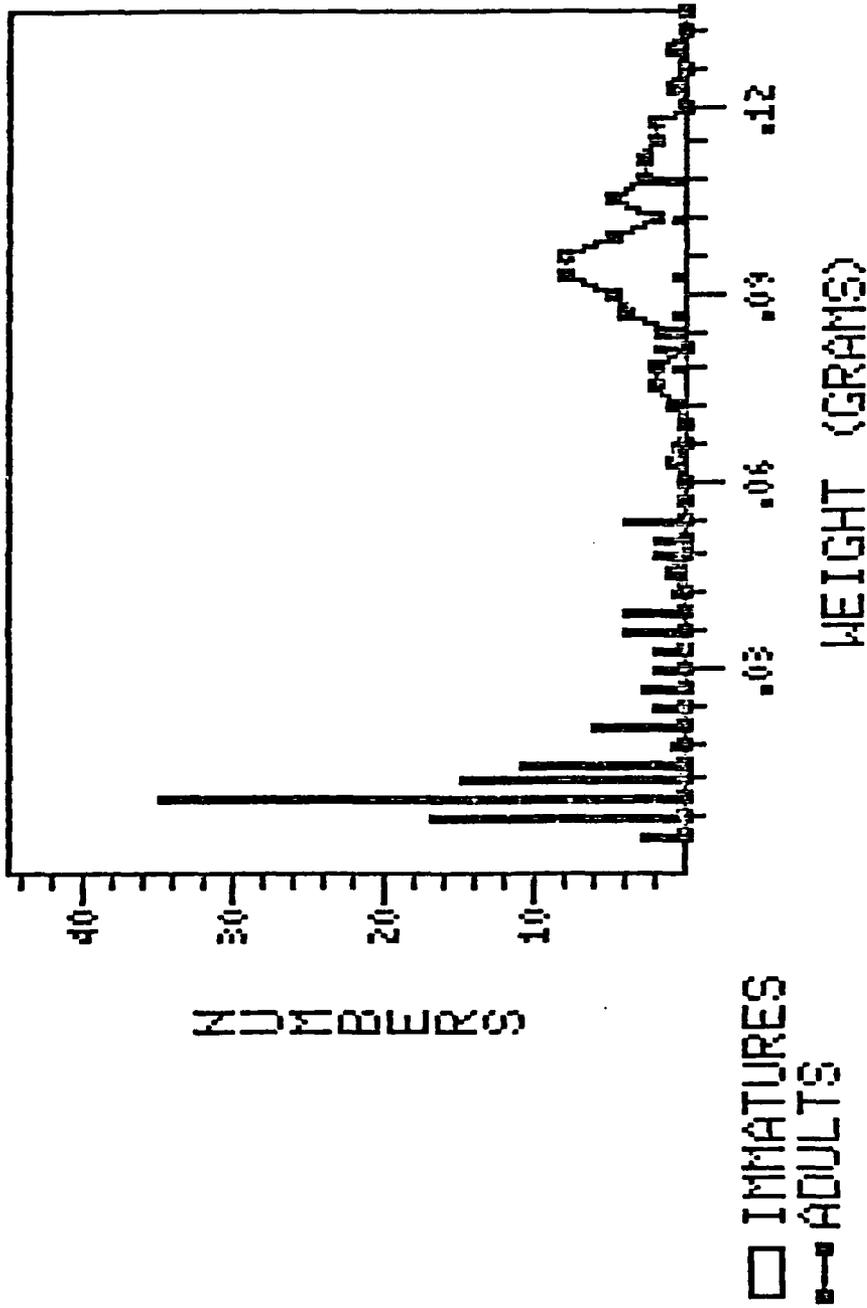


Fig. 78. Weight frequencies in September populations of Dendrobaena octaedra (1983 Test and Control samples combined).

WEIGHT CLASSES A. TURGIDA

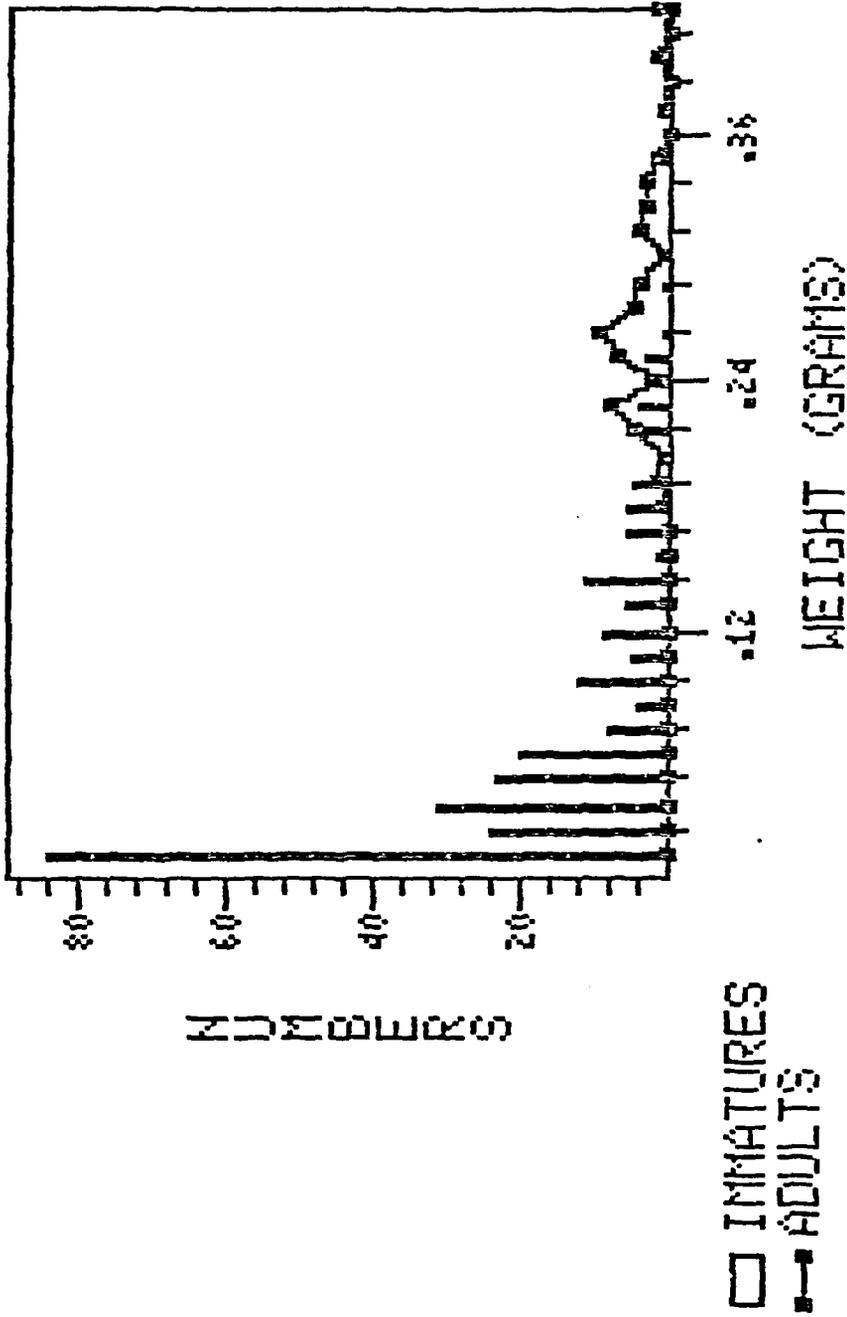


Fig. 79. Weight frequencies of September populations of Aporectodea turgida (1983 Test and Control samples combined).

WEIGHT CLASSES
A. TUBERCULATA

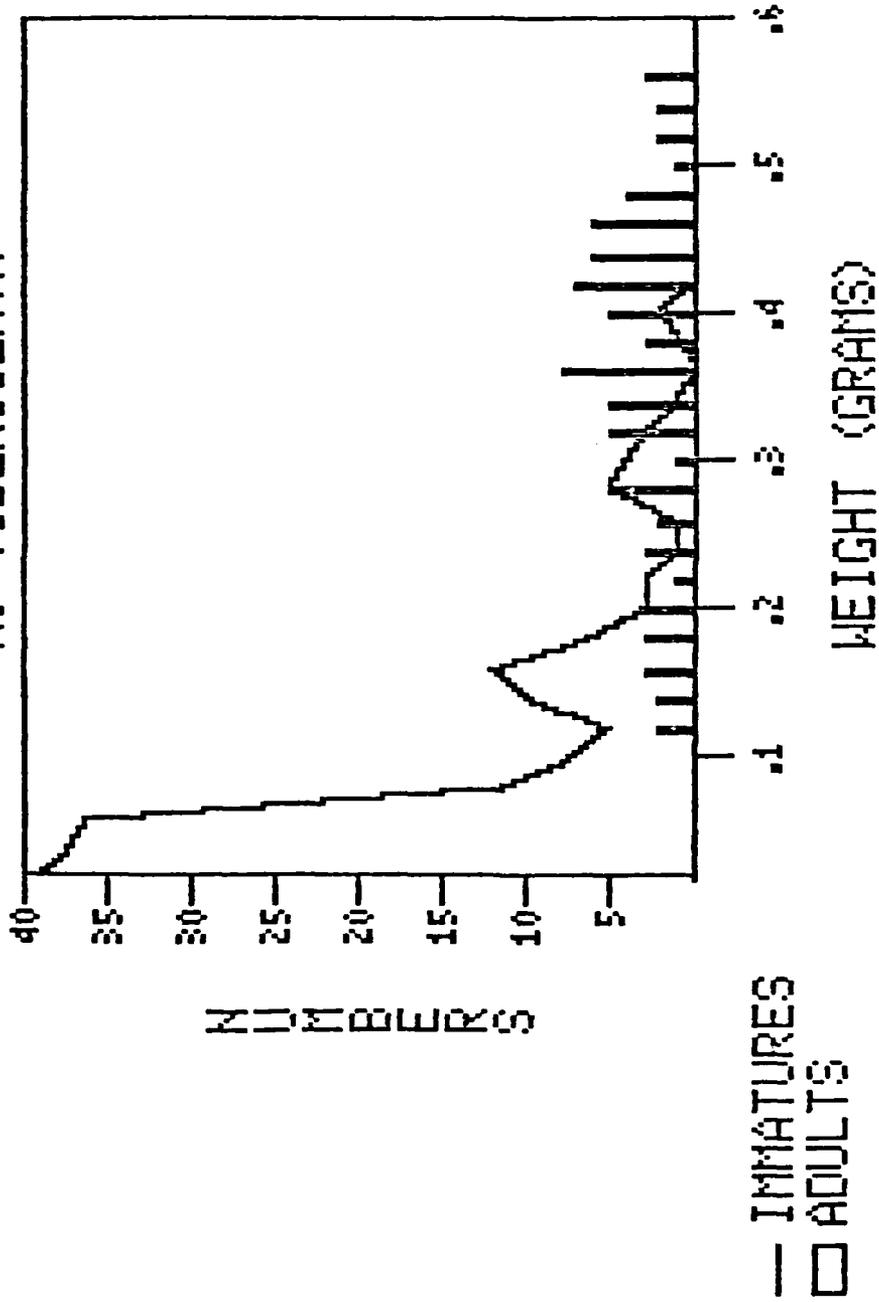


Fig. 80. Weight frequencies in September populations of Aporectodea tuberculata (1983 Test and Control samples combined).

the weeks preceding the September 1983 sampling date.

Table 33 provides a summary of mean weights per life stage for the four species. Between - site differences are not expected to exist, but will be tested once a full set of seasonal data is available. As postulated by Bouche (1977), the two endoges species, A. tuberculata and A. turgida, tend to be larger than the epiges. Of the latter, L. rubellus reaches weights similar to those of A. turgida (Table 33), but they are a great deal more variable. Further biological data will be needed to determine the relative functional position of L. rubellus within the lumbricid associations in Test and Control sites.

c. Spatial distribution and environmental parameters:

No site is completely homogeneous, and a possible correlation between quadrat and lumbricid population variation was analyzed. With only two sample dates available, the result was not significant, but potential quadrat-specific sources of variation will be further tested once a full season's set of data is at hand.

Species population densities were also not significantly correlated to humus depth and litter weight. Litter feeders, i.e. D. octaedra and L. rubellus (Reynolds 1977), can be expected to be most abundant where the substrate is most ample (Edwards & Lofty 1972). However, lack of correlation indicates that other environmental or behavioral parameters have overriding influence on population distribution.

August and September samples lent themselves well to analysis of species-specific depth distribution. The two weeks preceding the

Table 33: Mean weights in mg \pm SE, with coefficients of variation in parenthesis, of four earthworm species by stages (cocoons, immatures, and adults).

<u>Species</u>	<u>Cocoons</u>	<u>Immatures</u>	<u>Adults</u>
<u>D. octaedra</u> ¹⁾	3.53 \pm .05 (21%)	22.2 \pm 3.0 (151%)	93.7 \pm 3.4 (27%)
<u>L. rubellus</u> ²⁾	9.4 \pm 0.1	52.8 \pm 0.7 (117%)	306 \pm 105 (138%)
<u>A. tuberculata</u> ¹⁾	17 \pm 8 (45%)	85.4 \pm 9.0 (106%)	346 \pm 13 (35%)
<u>A. turgida</u> ²⁾	13.4 \pm 0.6 (34%)	62.5 \pm 4.5 (120%)	256 \pm 9.0 (27%)

1) specimens from September 14 Control samples

2) specimens from September 11 Test samples

August sampling date were drier and hotter than those preceding the September sampling date, and these temperature/moisture differences were found to be reflected in vertical distribution patterns of certain species.

Both epiges, D. octaedra and L. rubellus, frequented the litter layer when moisture was adequate, but retreated into the humus under dry conditions (Fig. 81 and 82). For both species, vertical distribution differences were highly significant (G test, $P < 0.001$). Endoges, A. turgida (Fig. 83) and A. tuberculata (Fig. 84) were distributed about equally between humus and deeper soil layers. In accordance with observations by Rundgren (1975) and Gerard (1967), vertical redistribution in response to moisture stress was much less pronounced in these endogens than in epiges. Distribution of A. turgida in the soil profile exhibited no significant seasonal differences. A. tuberculata showed a slight response to increased moisture (G test, $P < 0.05$) by migrating into the humus layer in September (Fig. 84).

4.3. Lumbricid populations at Turner Rd.

The ambiguous location of the Turner Rd. site with respect to the antenna corridor prompted us to abandon the site in late 1983. Data obtained there can be used, however, to validate the experimental design selected for long-term studies in definitive sites.

In 1982, sampling was performed according to the site's micro-relief (hills and depressions, 10 samples each). Totals obtained of each species, over three 1982 dates, are here used to describe the lumbricid association at Turner Rd. (Table 34): among endoges, O. tyrtaeum is dominant, A. tuberculata subdominant, E. rosea rare.

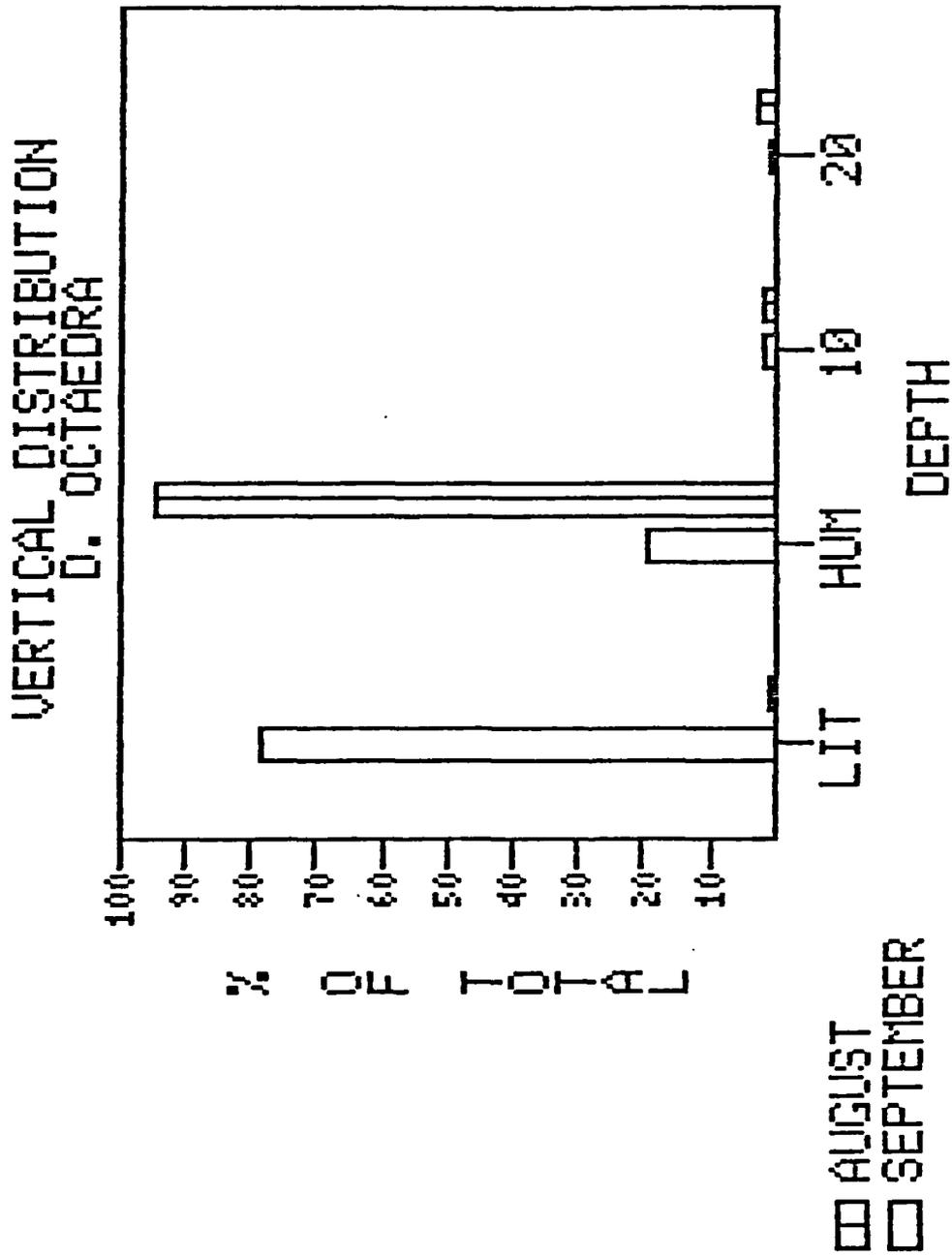


Fig. 81. Vertical distribution of Dendrobaena octaedra (immatures plus adults) in August and September (Test and Control samples combined).

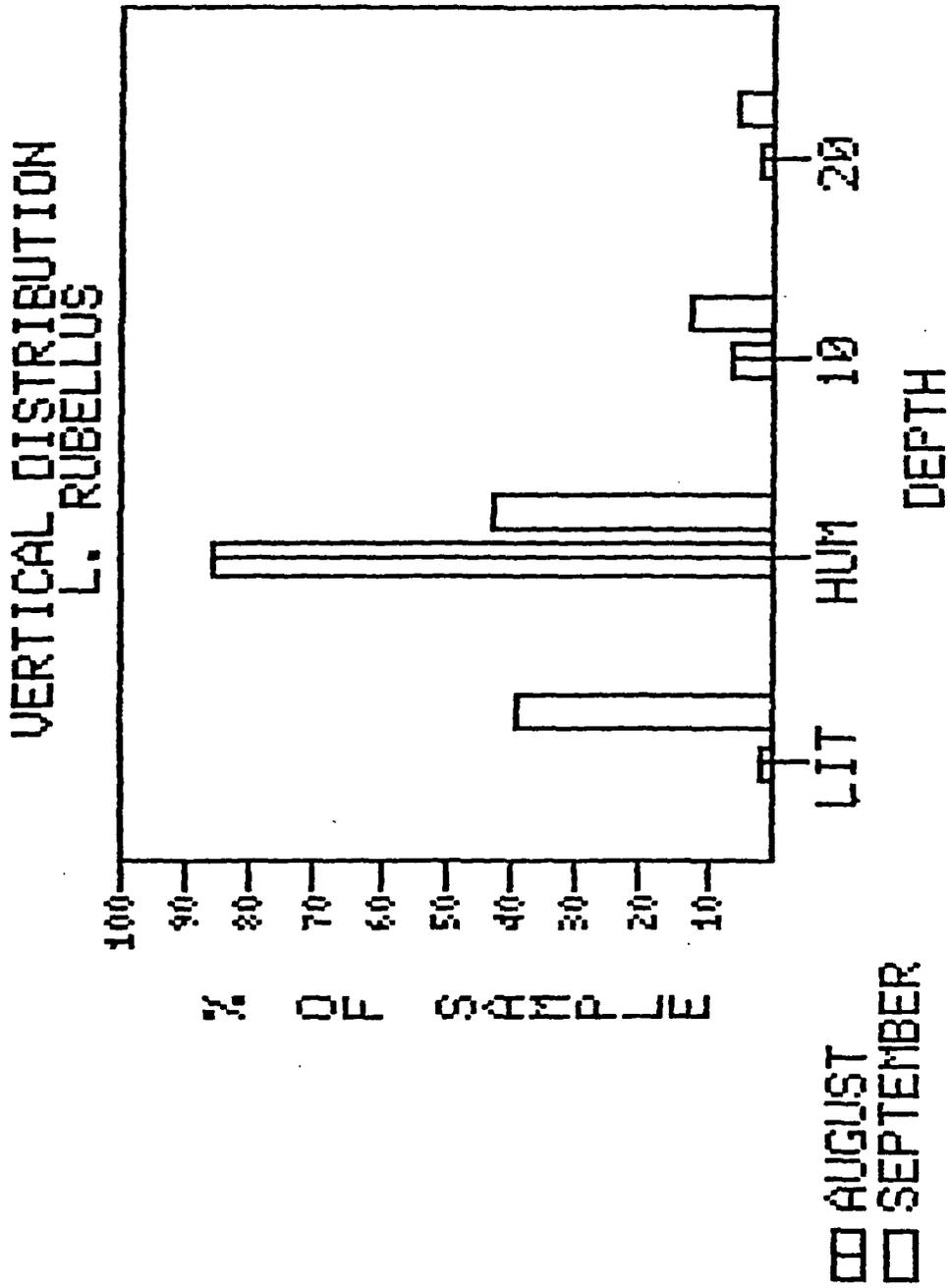


Fig. 82. Vertical distribution of *Lumbricus rubellus* (immatures plus adults) in August and September (Test and Control samples combined).

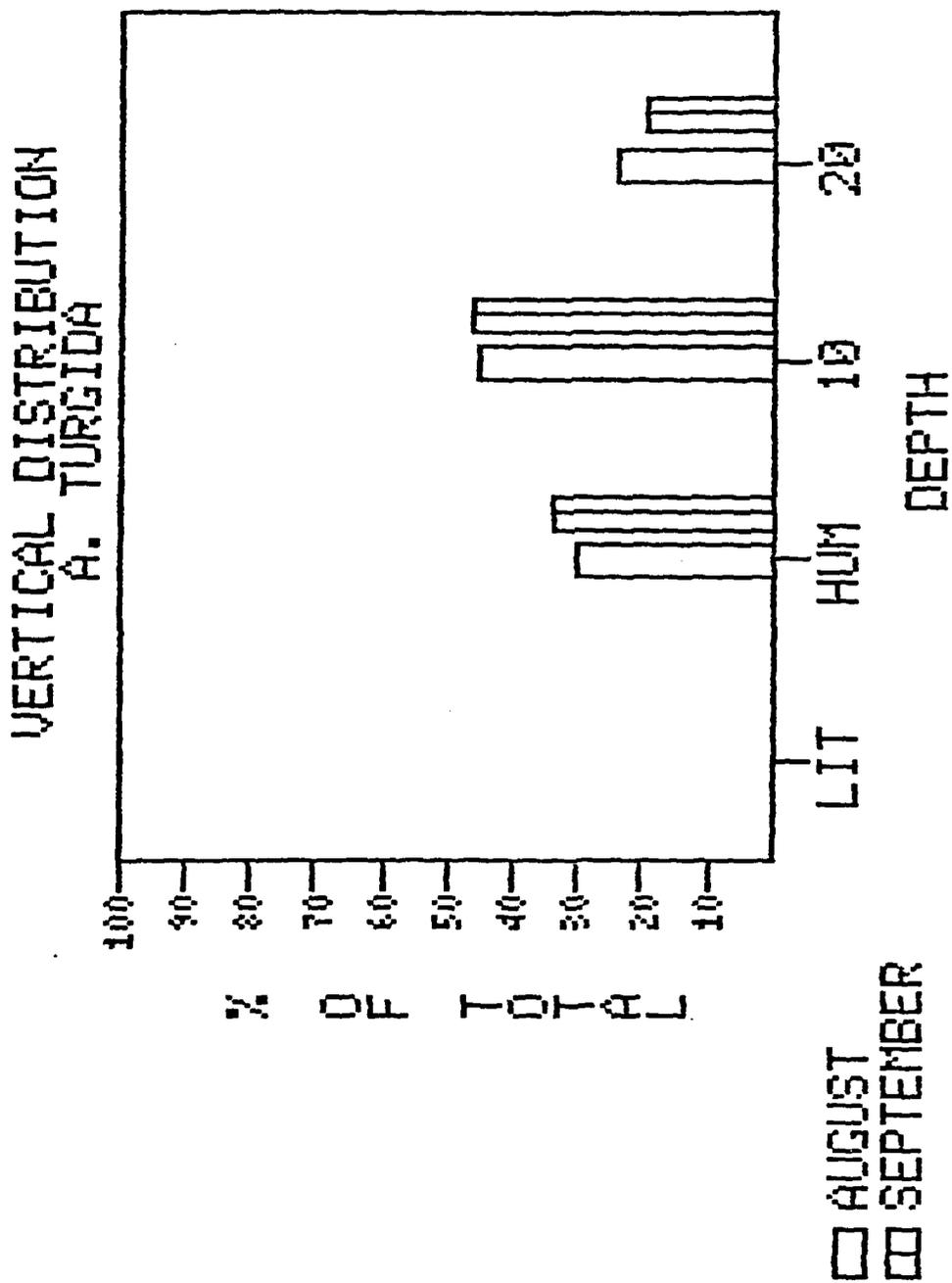


Fig. 83. Vertical distribution of *Aporrectodea turgida* (immatures plus adults) in August and September (Test and Control samples combined).

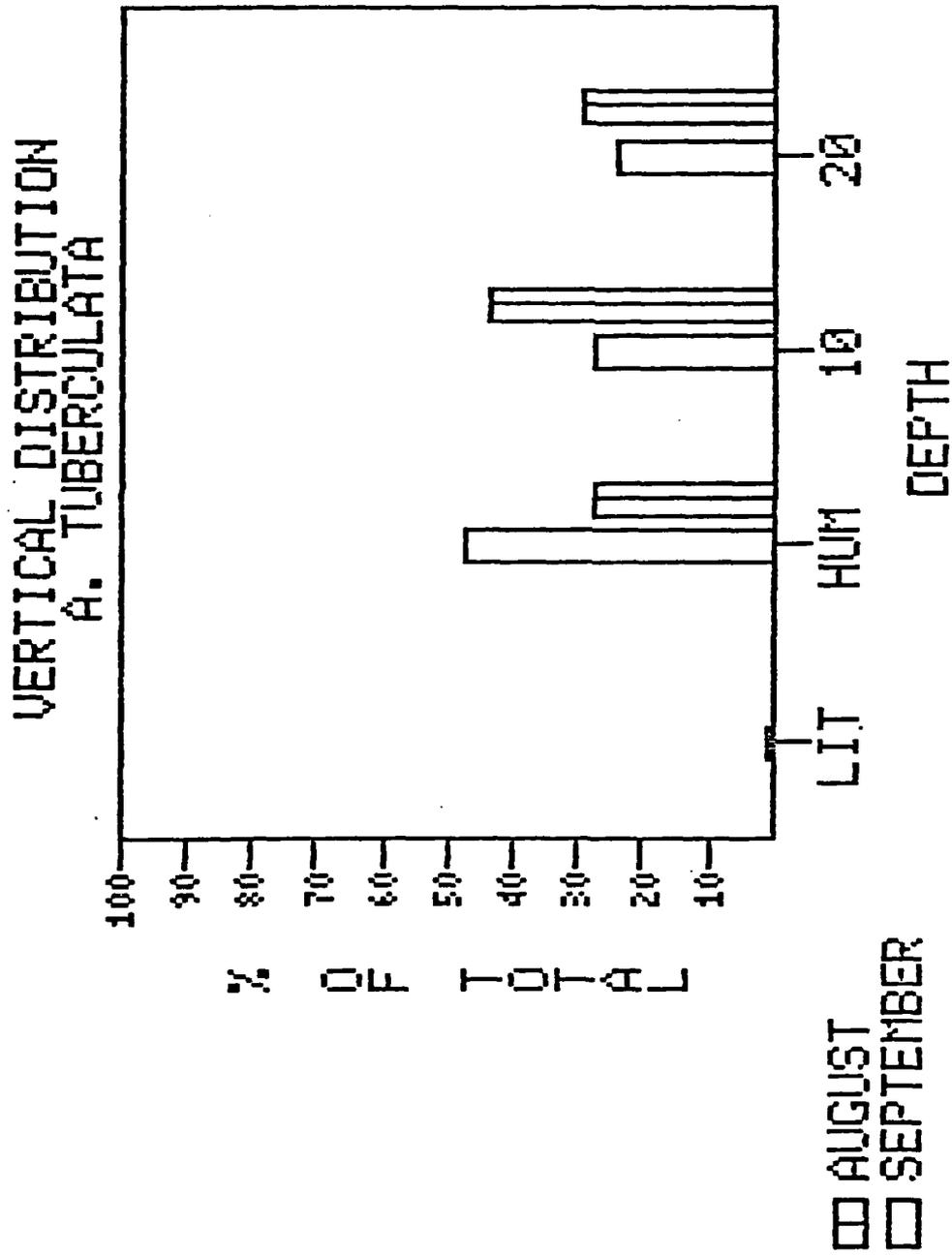


Fig. 84. Vertical distribution of *Aporectodea tuberculata* (immatures plus adults) in August and September (Test and Control samples combined).

Of the two epiges, D. octaedra is much more abundant than L. rubellus. The anecique guild is not represented at all. Total lumbricid density of approximately 330/m² is somewhat lower than in definitive sites, but must be considered an underestimate: litter subsamples were not included, and, more importantly, the efficient wet-sieving method had not been instituted at that time.

Table 34. Combined data from 3 sampling dates at Turner Rd., 1982: mean (+ S.E.) population densities in Hill and Depression samples, and combined totals.

<u>Species</u>	<u>Hill</u>	<u>Depression</u>	<u>Total</u>
<u>D. octaedra</u>	125 + 27	113 + 31	119 + 21
<u>L. rubellus</u>	26 + 9	45 + 14	36 + 8
<u>O. tyrtaeum</u>	118 + 31	127 + 31	122 + 22
<u>A. tuberculata</u>	40 + 16	58 + 27	49 + 15
<u>E. rosea</u>	.5 + .5	1.7 + 1.2	1.1 + .6

Table 34 shows that topographic location was not an important source of variability in horizontal distribution. Differences between hill and depression samples were not significant, despite the fact that humus depth in depressions was almost double that on hummocks. These data confirm earlier conclusions, i.e. that humus depth and distribution patterns of lumbricids are not correlated to a measureable degree. In addition, we may infer that our sampling design in definitive sites is valid: although all sites possess small-scale topographic heterogeneity, random sampling according to

grid coordinates (described in section B), irrespective of relief, will yield accurate estimates of lumbricid populations dynamics.

For spring and summer of 1983, species population changes are illustrated in Figures 85 to 88. Here, too, we use the data to point out potential sources of variability and of error in interpretation, rather than taking results at their face value.

Sampling at Turner Rd. was random, i.e. samples were taken along "transects" through the site, distance between samples being determined as an arbitrarily chosen number of paces. On each date, the starting points of these transects were changed.

At the close of the season, it was discovered that some transects reached areas of the site which differed greatly in soil texture, slightly in canopy cover, and greatly with respect to lumbricid populations. This was confirmed through a soil type survey by W. Summers (Soil Conservation Service) as well as through a non-quantitative, on-site, survey of lumbricid species distribution.

In short, we suspect that Figures 85 to 88 reflect, to an unknown extent, the effect of site heterogeneity on horizontal population distribution, and not true seasonal numerical fluctuations. L. rubellus and A. tuberculata (Figs. 87 and 88) in particular, within a relatively short distance from the main site area, "lose" their relative abundance values within the species association.

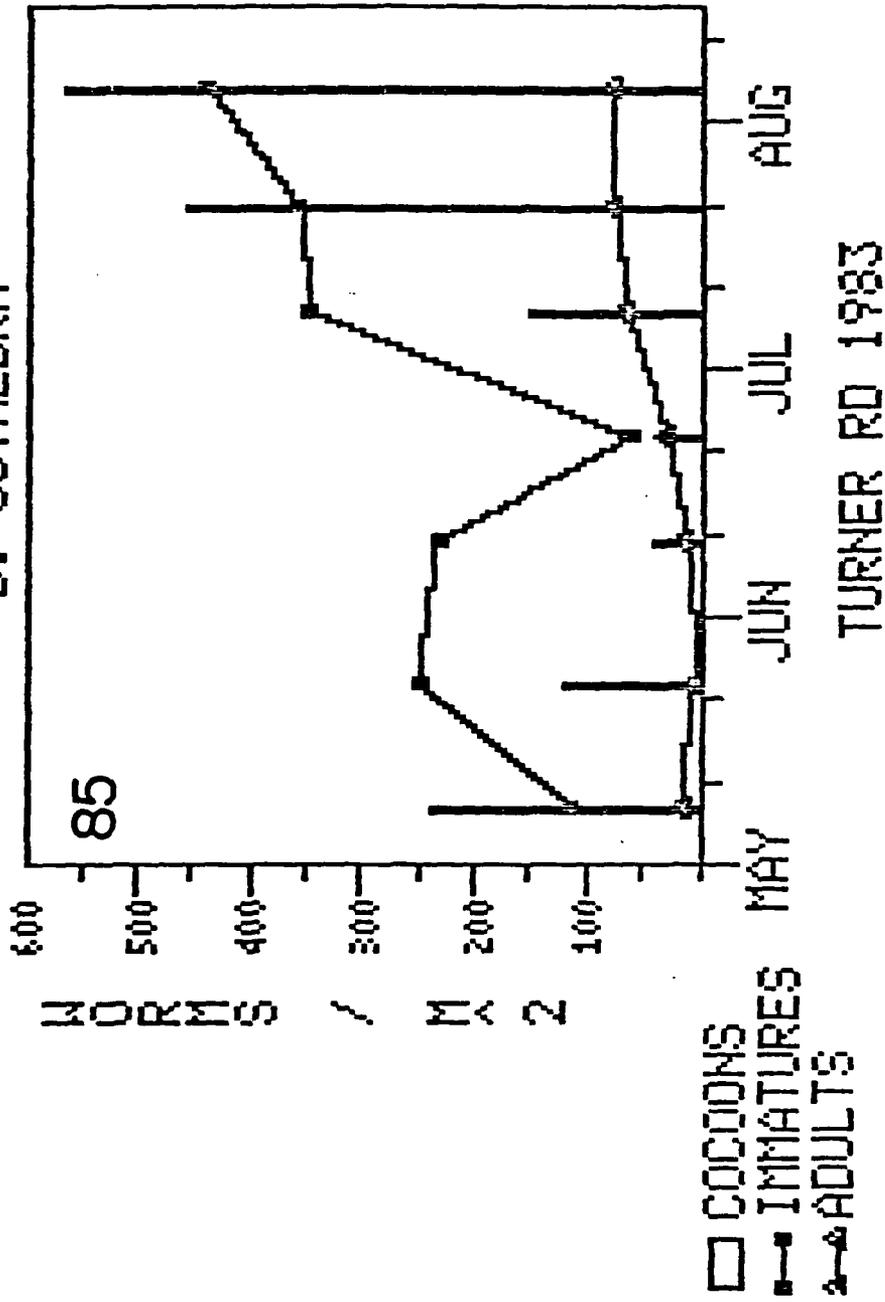
With respect to the lumbricid study program in Test and Control sites, we can now conclude that the grid system, dividing relatively large sites into permanent quadrats, is likely to be the best possible experimental approach. Recurrent sampling of the same quadrats on successive dates is combined with detailed quantification

of quadrat - specific vegetation and soil characteristics. As a result, population variability and site heterogeneity, should it exist, can be detected and analyzed (with respect to lumbricids, no such relationship has emerged so far).

Finally, Figures 85 to 88 also show cocoon densities, in order to illustrate the difference between handsorting and wet-sieving efficiencies; i.e., the apparent drastic increase in cocoon densities in mid-summer (e.g. Fig. 85) is a direct result of change in technique, and can not be interpreted as increased reproductive activity. Having used Turner Rd. material to optimize design and techniques, we can now be confident that our monitoring and sampling programs in definitive sites are well controlled.

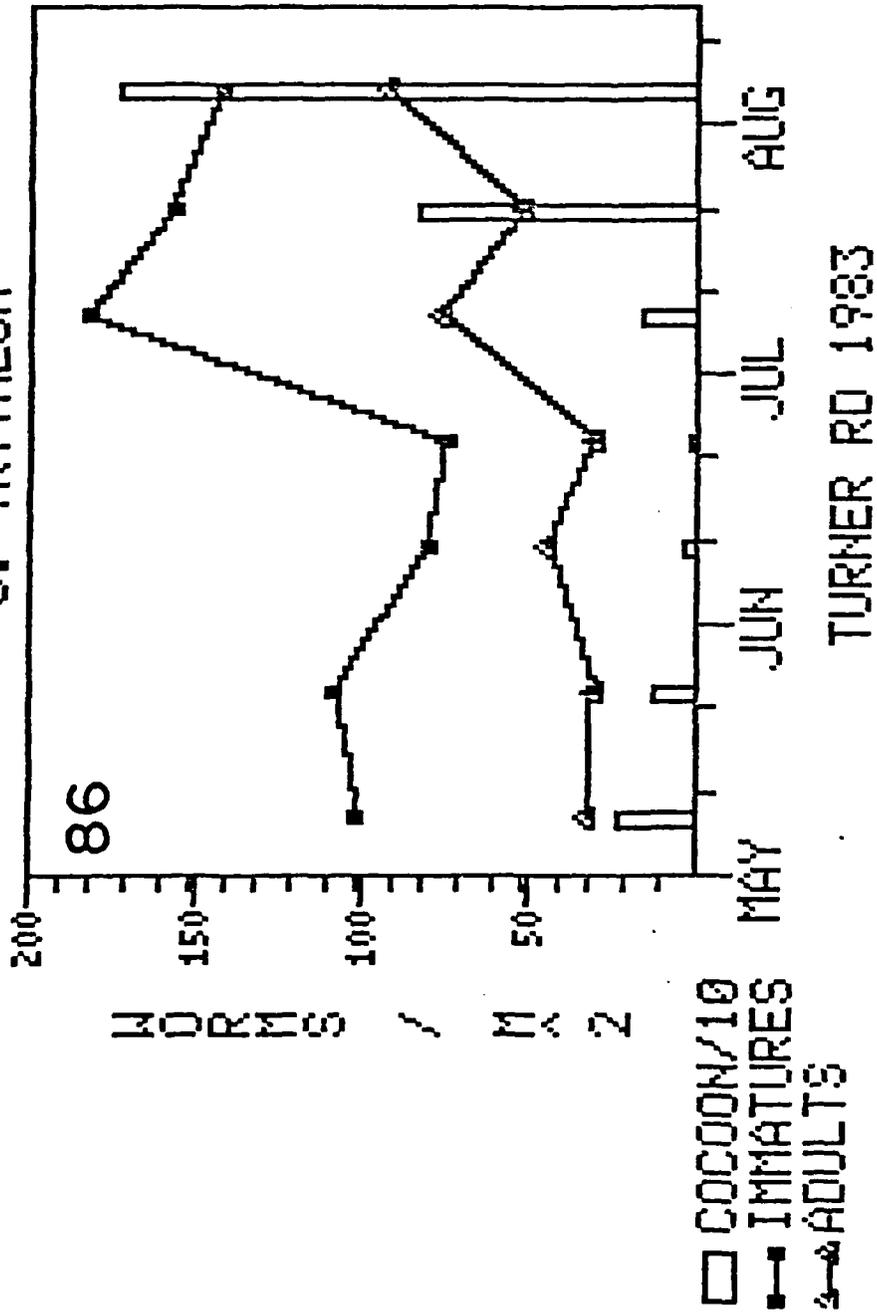
Figs. 85 - 88. Population density fluctuations, May to August, of four lumbricid species at Turner Rd, 1982 (n = 20 samples per date). The combined handsorting/wet-sieving method was initiated in late July.

EARTHWORM DENSITIES D. OCTAEDRA

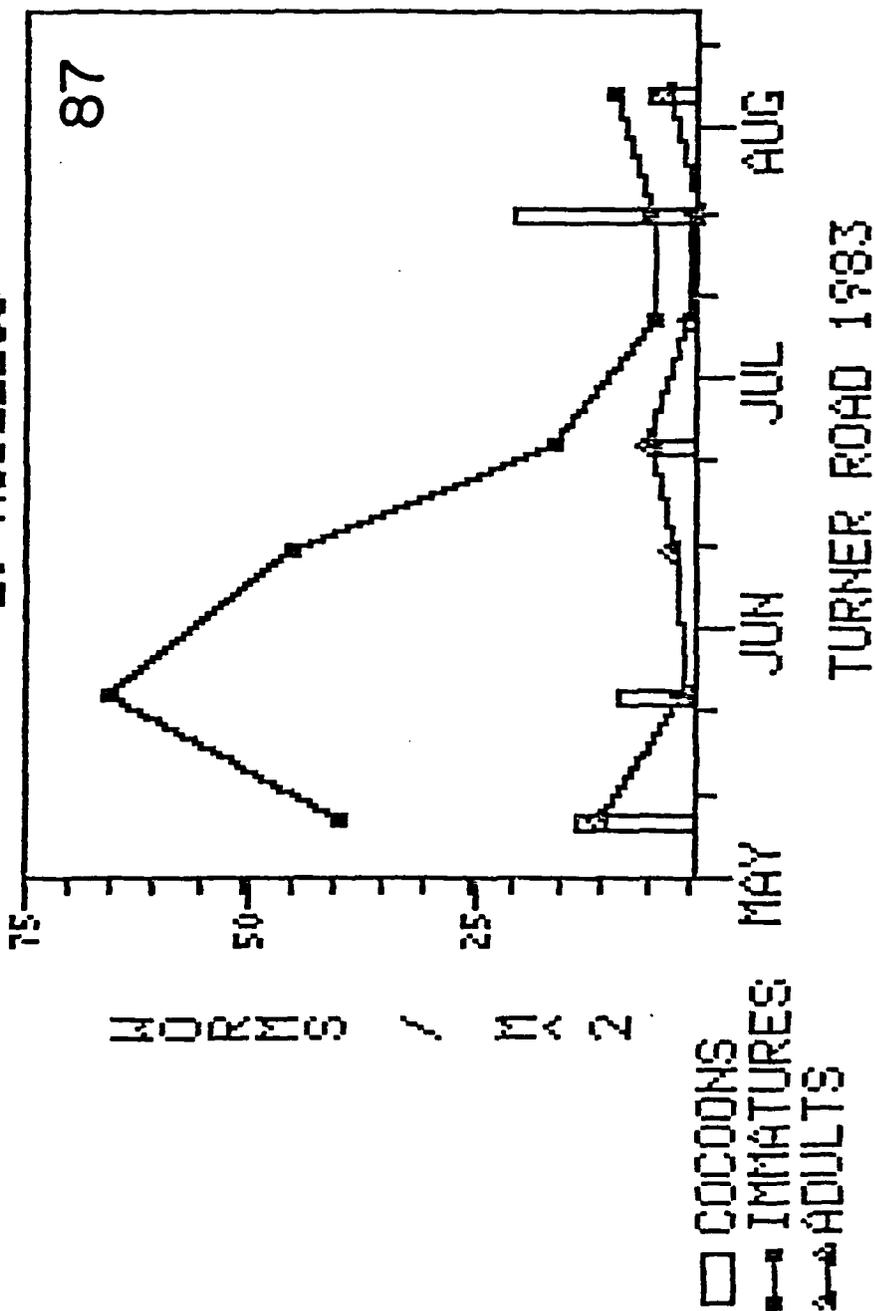


TURNER RD 1983

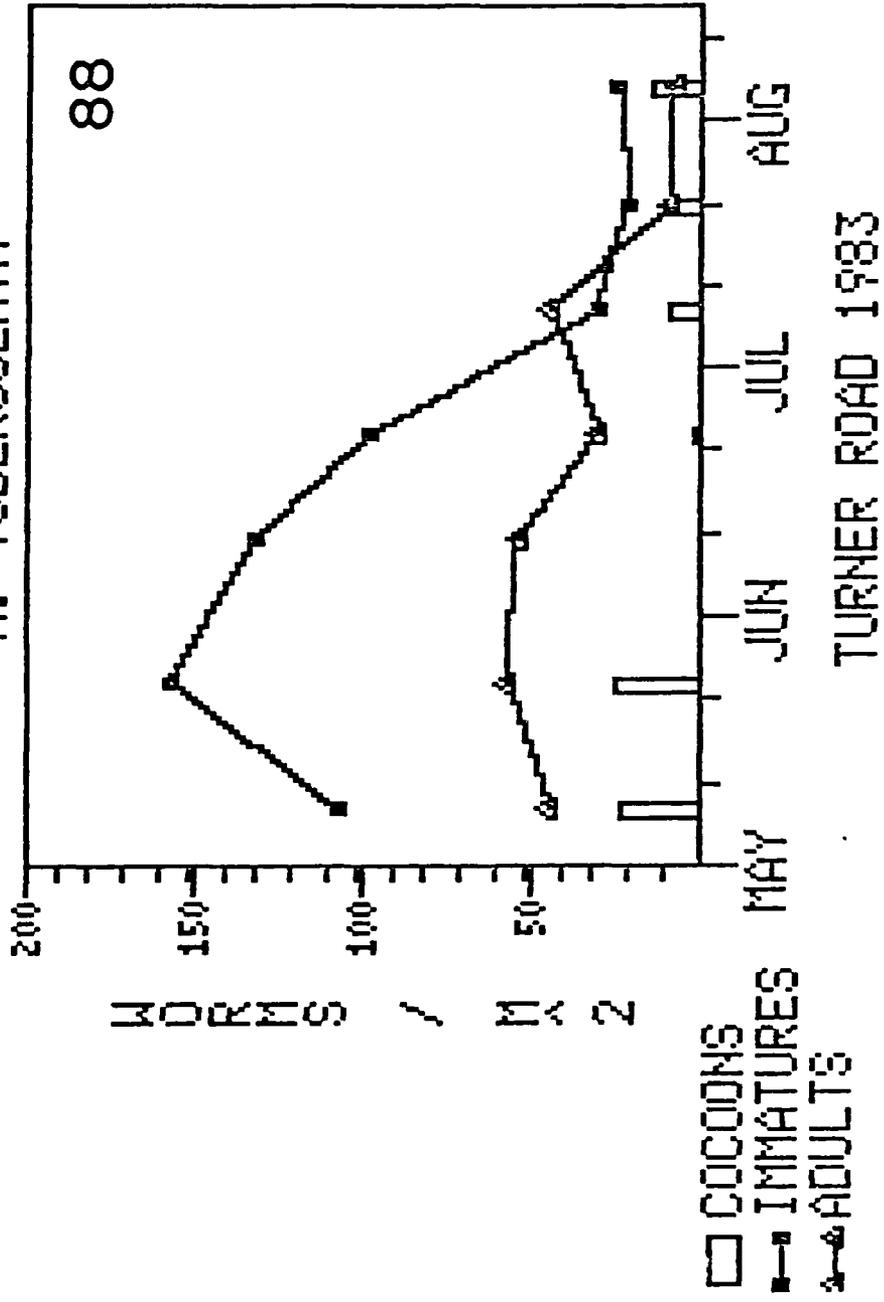
EARTHWORM DENSITIES
O. TRYPAEUM



EARTHWORM DENSITIES L. RUBELLUS



EARTHWORM DENSITIES
A. TUBERCULATA



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APPENDIX A

Checklist (subject to continued updating) of arthropod and lumbricid taxa so far encountered in the general ELF system area.

CHECKLIST
OF SOIL-LITTER ARTHROPODA AND EARTHWORMS
IN FORESTS IN THE ELF SYSTEM AREA

CLASS OLIGOCHAETA

ORDER HAPLOTAXIDA

Family Lumbricidae

- Aporrectodea longa (Ude)
A. tuberculata (Eisen)
A. turgida (Eisen)
Dendrobaena octaedra (Savigny)
Eisenia rosea (Savigny)
Lumbricus terrestris Linnaeus
L. rubellus Hoffmeister
Octolasion tyrtaeum (Savigny)

CLASS ARACHNIDA

ORDER PSEUDOSCORPIONIDA

Family Chthoniidae

- Mundochthonius rossi Hoff

Family Neobisiidae

- Microbisium confusum Hoff

ORDER ARANEAE

Family Theridiidae

- Ctenium riparius (Keyserling)
C. longipalpus Kaston
C. laticeps (Keyserling)

Family Linyphiidae

- Drapetisca sp.
Helophora sp.
Lepthyphantes sp.
Microneta sp.
Meioneta sp.
Bathyphantes pallida (Banks)
Centromerus persoluta (O.P.-Cambridge)
C. sylvaticus (Blackwell)
Macrargus multesimus (O.P.-Cambridge)

Family Micryphantidae

- Ceraticelus fissiceps (O.P.-Cambridge)

Family Agelenidae

- Wadotes calcaratus (Keyserling)
Cicurina brevis (Emerton)
C. robusta Simon
Agelenopsis kastoni Chamberlin and Ivie
Coras montanus (Emerton)

Family Hahniidae

- Neoantistea radula (Emerton)
Hahnia cinerea C.L. Koch

- Family Lycosidae
Pirata minutus Emerton
P. marxi Stone
P. maculatus Emerton
Lycosa gulosa Walckenaer
Trochosa terricola Thorell
Tarentula sp.
- Family Clubionidae
Agroeca emertoni Kaston
Phrurotimpus borealis (Emerton)
P. similis Banks
Ciracanthium sp.
- Family Thomisidae
Oxyptila sp.
- Family Amaurobiidae
Callioplus borealis (Emerton)
- Family Philodromidae
Philodromus sp.
- Family Epeiridae
Epeira displicata Hentz
- Family Dictynidae
Scotolathys pallidus (Marx)
- Family Salticidae
Hentzia sp.
- Family Araneidae
Araniella displicata (Hentz)
- Family Plectreuridae
 Family Homalonychidae

ORDER OPILIONES

- Family Phalangiidae
Caddo boopis Crosby
Odiellus pictus (Wood)
Leiobunum nigripes (Weed)
L. politum Weed
- Family Ischyropsalidae
Sabacon crassipalpi (L. Koch)
- Family Nemastomatidae
Crosbycus dasycnemus (Crosby)

ORDER ACARINA

- Suborder Oribatida
 Suborder Mesostigmata
 Family Veigaiidae
 Parasitidae
 Macrochelidae
 Eviphididae
 Phytoseiidae
 Ascidae
 Laelapidae
 Digamasellidae
 Rhodacaridae
 Ologamasidae

- Uropodidae
- Zerconidae
- Ameroseiidae
- Parholaspididae
- Epicriidae
- Sejidae
- Suborder Prostigmata
- Family Eupodidae
- Rhagidiidae
- Bdellidae
- Cunaxidae
- Scutacaridae
- Nanorchestidae
- Ereynetidae
- Tydaeidae
- Pygmephoridae
- Tarsonemidae
- Johnstonianidae
- Tetranychidae
- Trombiculidae
- Penthaleidae
- Pachygnathidae
- Anystidae
- Pyemotidae
- Alicorhagiidae
- Adamystidae
- Cheyletidae
- Alycidae
- Suborder Astigmata
- Family Acaridae
- Glycyphagidae
- Histiostomatidae
- Saproglygyphagidae
- Echimyopodidae

CLASS CHILOPODA

- ORDER GEOPHILOMORPHA
- Family Geophilidae
- Strigamia chionophila Wood
- S. branneri Bollman
- Taiyuna opita Chamberlin
- ORDER LITHOBIOMORPHA
- Family Lithobiidae
- Nadabius iowensis (Meinert)
- Lithobius forficatus Linnaeus

CLASS DIPLOPODA

ORDER POLYZONIIDA

Family Polyzoziidae

Polyzonium bivirgatum (Wood)

ORDER JULIDA

Family Parajulidae

Uroblaniulus canadensis (Newport)

ORDER CHORDEUMATIDA

Family Cleidogonidae

Cleidogona exaspera Williams and HefnerCLASS INSECTA

ORDER PROTURA

Family Eosentomidae

Eosentomon pallidum Ewing

ORDER COLLEMBOLA

Family Hypogastruridae

Anurida furcifera (Mills)A. pygmaea (Börner)Friesea sublimis MacnamaraWillemia denisi MillsW. intermedia MillsW. similis MillsNeanura muscorum (Templeton)Odontella substriata WrayXenylla acauda GisinPseudachorutes sp.

Family Onychiuridae

Tullbergia granulata MillsT. mala Christiansen and BellingerOnychiurus affinis AgrenO. parvicornis MillsO. similis Folsom

Family Isotomidae

Proisotoma minima (Absolon)Isotomiella minor (Schaeffer)Folsomia nivalis (Packard)Anurophorus binoculatus (Kneseman)Isotoma nigrifrons FolsomI. notabilis SchaefferI. viridis Fourlet

Family Entomobryidae

Tomocerus flavescens Tullberg
T. lamelliferus Mills
Orchesella hexfasciata Harvey
O. ainslei Folsom
Lepidocyrtus helenae Snider
L. hirtus Christiansen and Bellinger
L. lignorum (Fabricius)
L. paradoxus Uzel
L. violaceus Fourcroy
L. unifasciatus James
Entomobrya assuta Folsom
E. clitellaria Guthrie
E. multifasciata (Tullberg)
E. nivalis (Linne)
E. comparata Folsom
E. purpurescens (Packard)
Pseudosinella violenta (Folsom)

Family Neelidae

Neelus minimus (Willem)
N. tristani (Denis)
N. minutus (Folsom)
N. snideri (Bernard)

Family Sminthuridae

Sminthurides lepus Mills
Sminthurinus henshawi (Folsom)
S. conchyliatus Snider
S. quadrimaculatus (Ryder)
S. intermedius Snider
S. butcheri Snider
S. nigromaculatus Tullberg
Dicyrtoma marmorata (Packard)
Arrhopalites amarus Christiansen
A. benitus (Folsom)
Bourletiella hortensis (Fitch)

ORDER ORTHOPTERA

Family Gryllacrididae

Ceutophilus brevipes Scudder
C. maculatus (Harris)

ORDER PSOCOPTERA

Family Polypsocidae

Epipsocidae
 Psocidae
 Pseudocaeciliidae

ORDER COLEOPTERA

Family Carabidae

Pterostichus melanarius IlligerP. coracinus NewmanP. pennsylvanicus LeConteP. adstrictus EschscholtzP. adoxus SayP. novus StraneoP. relictus NewmanCalathus ingratus DejeanC. gregarius SaySynuchus impunctatus SayCymindis cribricollis DejeanHarpalus fuliginosus DuftschmidAgonum retractum LeConteA. placidum SayClivina fossor LinneMyas cyanescens DejeanBembidion quadrimaculatum oppositum SayTrechus obtusus SayCarabus sylvosus SayNotiophilus aenus Herbst

Family Scarabaeidae

Geotrupes sp.

Family Staphylinidae

Leiodidae

Cryptophagidae

Curculionidae

Silphidae

Chrysomelidae

Endonychidae

Lathridiidae

Pselaphidae

Eucnemidae

Nitidulidae

Lampyridae

Elateridae

Scaphidiidae

Melandryidae

Pedilidae

Cerambycidae

Byrrhidae

Scydmaenidae

Languriidae

ORDER DIPTERA

Family Cecidomyidae
Sciaridae
Phoridae
Tipulidae
Psychodidae
Chironomidae
Mycetophilidae
Drosophilidae
Bibionidae
Culicidae
Dolichopodidae
Heleomyzidae
Sciomyzidae
Sphaeroceridae
Muscidae
Tachinidae

APPENDIX B

List of new geographic distribution records for arthropod species so far encountered in the ELF system area, organized as a geographic hierarchy (USA to Dickinson County).

A. New distribution record for U.S.A.:

Collembola:

Sminthurinus nigromaculatusB. New records for Michigan:

Collembola:

Sminthurinus conchyliatusS. intermediusArrhopalites amarusLepidocyrtus hirtusEntomobrya comparataAnurophorus binoculatusTullbergia malaOnychiurus affinisO. parvicornisO. similisAnurida furciferaFriesea sublimisWillemia denisiW. similisOdontella substriataXenylla acaudaParanura anopsNeelus minimusN. tristani

Chilopoda:

Strigamia branneriTaiyuna opitaC. New records for Upper Peninsula:

Collembola:

Sminthurus butcheriTomocerus lamelliferusLepidocyrtus paradoxusProisotoma minimaIsotomeilla minorIsotoma nigrifronsI. notabilisTullbergia granulataAnurida pygmaeaWillemia intermediaNeelus minutusNeelus snideri

Opiliones:

Caddo boopis

Diplopoda:

Cleidagona exaspera

D. New records for Dickinson County:Collembola:

Sminthurides lepus
Sminthurinus henshawi
Sminthurus quadrimaculatus
Dicyrtoma marmorata
Arrhopalites benitus
Bourletiella hortensis
Tomocerus flavescens
Orchesella hexfasciata
Lepidocyrtus helenae
L. lignorus
L. violaceus
L. unifasciatus
Entomobrya assuta
E. clitellaria
E. multifasciata
E. nivalis
E. purpurascens
Isotoma viridis
Neanura muscarum

Diplopoda:

Uroblaniulus canadensis

Coleoptera (Carabidae):

Bembidion quadrimaculatum oppositum
Pterostichus adstrictus
P. coracinus
P. novus
P. relictus
Calathus ingratus
Synuchus impunctatus
Agonum placidum
Cymindis cribricollis
Clivina fossor